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Papers from the Institute of Nutrition of Central America and Panama

Introduction

THE Institute of Nutrition of Central America and Panama (INCAP) was inaugurated in 1949 to determine the nutrition problems of its member countries, to find practical means of approaching their solution and to help the countries to apply these measures. It began with active participation of Guatemala, El Salvador and Honduras; Costa Rica and Panama became active members in 1950 and 1951, respectively, and Nicaragua in 1954.

The W. K. Kellogg Foundation was asked to assist in the founding of this new institute and did so not only by furnishing fellowships for training of personnel and funds for the purchase of basic equipment, but also by making a three-year grant to the Pan American Sanitary Bureau (PASB) to establish a nutrition section whose chief would also serve as the director of the new institute. The member countries requested the Pan American Sanitary Bureau, which shortly afterwards became also the regional office for the Americas of the newly established World Health Organization (WHO) to serve as the administrative agency for INCAP. A representative of the Ministry of Health of each member country and of PASB formed the Directing Council, and the basic budget was provided by equal annual quota contributions from each country. Each member country has also established a nutrition section in its Health Department staffed with trained personnel for the development of applied nutrition programs. The central Instituto was located in Guatemala City in a new building constructed by the government of Guatemala.

To determine the nutrition problems of the area, surveys of the dietary habits and nutritional status of the various socioeconomic groups within each member country were initiated. These in turn required laboratory facilities for the analysis of foods, the detection of parasites and anemias, and the biochemical evaluation of nutriture. As specific problems such as endemic goiter, kwashiorkor, marasmus and avitaminosis A were identified, and the extent of chronic protein malnutrition and retarded growth and development in preschool children were recognized, increasing emphasis was placed on studying these specific questions and developing practical approaches to their prevention. Inevitably, INCAP has also become deeply involved in studies of the relationships between nutrition and infection, the development of protein rich foods, specific amino acid requirements and interrelationship, amino acid supplementation of vegetable proteins and the relationship between diet and atherosclerosis.

As data became available from the surveys and other research activities, the application of this knowledge, through both training programs and the preparation of basic educational material, increased in scope and importance. Consultant and advisory service to both governmental and nongovernmental action

agencies within the member countries has also been a major activity.

Although INCAP was established for the countries of Central America and Panama, it early became important as a demonstration and training center for persons from all parts of Latin America and from many from other technically underdeveloped areas of the world. Last year, a formal two year course was initiated to prepare university graduates for the professions of dietitian, public health nutritionist and nutritionist in agricultural and education programs. It includes one year of academic study and one of internship. An intensive summer course for physicians with an advanced degree in public health was also inaugurated to be given alternatively in English and Spanish. It includes public health nutrition, evaluation of nutritional status and discussion of clinical nutrition problems from the point of view of public health and preventive medicine. In cooperation with FAO, a center for training in the technics of dietary surveys has also been established. Graduate research opportunities are also offered in all of the various major fields of INCAP activity.

Basic to the development of INCAP has been the policy of recruiting the professional staff from the member countries and arranging for them to receive thorough advanced training outside of the area. The sixteen senior members have a present total of fourteen Master's Degrees, six Ph.D.'s and four are physicians who are "board eligible" in their specialities. The results of this training policy are apparent not only in the demands on INCAP staff for consultant services to other areas but also in the scientific productivity. The current publications list includes 185 scientific articles in English, 255 in Spanish (including republication in Spanish of all articles appearing originally in English), 3 monographs, 30 student theses for various university degrees and nearly 150 educational and miscellaneous publications. INCAP is housed in a modern three story building in Guatemala City and administratively is divided into divisions of Public Health, Epidemiology, Statistics and Documentation, Physiological Chemistry, Agricultural and Food Chemistry, Clinical Investigation, and Administration.

Its library receives over 200 journals, has approximately 25,000 reprints and an extensive collection of documents of UN agencies and miscellaneous publications in nutrition.

The articles which have been selected for this special INCAP issue reflect the varied lines of research now actively in progress in INCAP with the support, not only of the member countries and PASB, but also of the National Institutes of Health, the Nutrition Foundation, Inc. Williams-Waterman Fund of the Research Corporation, the National Research Council, National Live Stock and Meat Board, the Rockefeller Foundation and such organizations as the E. I. du Pont de Nemours & Company, the Quaker Oats Company, Parke, Davis & Company, and Nestlé. They illustrate the use of biochemical measures for the evaluation of nutritional status, a nutrition survey in an unusual rural population, studies of the effect of environmental factors, including diet on serum cholesterol levels, the effect of infections on nitrogen metabolism, fundamental biochemical investigations into the nature of kwashiorkor and marasmus, and the clinical evaluation of low cost vegetable mixtures of high nutritive value developed for the prevention of protein malnutrition. Because of the clinical nature of this journal the extensive work of INCAP in the area of agricultural and food chemistry is not directly represented.

It has been a great privilege for me to serve as Director of INCAP since its inauguration and now to have the opportunity as guest editor to present a group of eleven papers illustrating some of INCAP's clinical research activities. Investigations of this type carry with them the double stimulus of acquiring new knowledge and contributing directly to the practical solution of the urgent nutrition problems of technically underdeveloped areas. It is hoped that these papers will give an idea of the variety of fundamental problems in the field of clinical and public health nutrition which require further research.

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Factors Influencing Serum Cholesterol Levels of Central American Children

I. Effect of Adding Fat and Animal Protein to the Diet

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THE rural population of Central America has been shown to have relatively low serum cholesterol levels,[1-3] a low dietary intake of both fat and animal protein,[1-3] and a very low prevalence of severe aortic atherosclerosis[4,5] and myocardial infarction.[6] Although at present there is no conclusive evidence that these phenomena are more than secondarily associated, attention has naturally been directed to the extent to which they are causally related. Although marked differences in serum cholesterol levels between socioeconomic and dietary groups within Guatemala are clearly evident, even among children of school age,[3] differences in the severity of aortic atherosclerosis cannot be detected until the fourth decade of life.[4,5] Differences in activity and psychologic stress have been postulated as accounting for some of the differences observed among adult socioeconomic groups, but this is less likely to be true for children.

Since the diets of rural Guatemalan school children are known to be extremely low in both fat and protein of animal origin, supplementary snacks offer a particularly convenient method of testing the effect of relatively long-term dietary changes on serum lipid levels. In view of the extensive evidence for the influence of dietary fat on serum cholesterol levels[7-9] and the suggestion that under certain circumstances methionine may be a factor affecting serum cholesterol levels,[9] arrangements were made to give groups of rural school children in different villages supplements designed to provide either milk or one of three different kinds of fat daily, for periods ranging up to nearly a year.

MATERIALS AND METHODS

Two rural villages in the highlands of Guatemala were selected for the administration of skim milk. Forty-one school children in Ciudad Vieja (CVS) and sixty in Santo Tomás Milpas Altas (STM) were given 60 gm. of reconstituted dried skim milk powder daily for twenty-eight weeks as a supplement to the customary diet. All these children were of predominantly Mayan Indian racial origin. The supplement provided approximately 200 calories per day and 20 gm. of protein. Blood samples were taken at the beginning of the trial and at the eighteenth and twenty-eighth week. The variation in number of children sampled at each examination in this and subsequent trials was due largely to the difficulty of obtaining permission to take a blood sample from a rural Indian child.

In the rural village of San Lorenzo el Cubo (SLC) and Santa María Cauqué (SMC), 30 gm. of fat were administered by spreading a fried black bean paste on a fried tortilla and serving it twice daily as a between meals snack

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TABLE I

Average Daily Dietary Intake of Eighty-one Guatemalan Children in 4 Rural Highland Towns*

Nutrients	Intake (gm.)	% of Calories	Nutrients	Intake (mg.)
Calories	1,581		Calcium	980
Animal protein	5.1	1.3	Phosphorus	988
Total protein	46.9	11.9	Iron	17.2
Carbohydrates	322	81.5	Thiamine	1.05
Total fat	13.8	7.9	Riboflavin	0.54
Fatty acids			Niacin	8.53
Saturated	2.6	1.5	Vitamin C	34.00
Oleic acid	5.8	3.3	Vitamin A	2,520†
Polyunsaturated	4.0	2.3		

Fatty Acid Content of Daily Supplements

	Saturated (gm.)	Oleic (gm.)	Polyunsaturated (gm.)
Skimmed milk (60 gm.)	0.6	0.3	0.1
Lard (30 gm.)	12.0	13.1	3.4
Cottonseed oil (30 gm.)	7.1	7.1	14.3
Hydrogenated cottonseed oil (30 gm.)	6.9	13.6	8.0

* SMC and three similar nearby towns not included in the experimental study.
† Expressed in international units.

providing a total of approximately 400 calories daily. In this way, recipients were not aware of differences in the type of fat used. In SLC a group of nineteen school children (group A) were given lard for forty-five weeks. Blood samples were taken at the beginning of the trial and at the nineteenth, thirty-seventh and forty-fifth week. In the same village of SLC, a second group of twenty-seven children (group B) were given hydrogenated cottonseed oil for twenty-six weeks. Blood samples were taken at the start of the trial and at the fifteenth and twenty-sixth week. In Santa María Cauqué (SMC) a group of 105 school children (group C) were given plain cottonseed oil for an eighteen-week period, and blood samples taken only at the beginning and end of the experiment.

Serum cholesterol levels were determined by the method of Abell et al.[10] The statistical analysis was made using the t test to evaluate the significance of the differences in means, and also the significance of the differences in paired data for individual children.

The average daily intake of eighty-seven school children in four nearby rural localities including one of the towns of the present study (SMC), and the composition of the supplements given, are presented in Table I. The food composition tables of INCAP[11] were used in determining the nutritional value and those of Hardinge and Crook[12] to calculate the fatty acid composition of the food consumed.

RESULTS

As shown in Table II, the daily administration of 60 gm. of skim milk during twenty-eight weeks to the school children of the rural villages of CVS and STM failed to produce any significant effect on serum cholesterol. Similarly, the data in Table III indicate that the daily administration of 30 gm. of either lard, hydrogenated cottonseed or plain cottonseed oil also failed to cause any change in serum cholesterol levels among the three groups. There was no significant correlation between serum cholesterol values and either age or gain in weight.

TABLE II

Serum Cholesterol Levels of Guatemalan School Children
(Effect of Daily Administration of 60 Gm. Skim Milk)

Period	No. of Children	Mean (mg./100 ml.)	Standard Deviation
Village CVS			
Initial	41	121	22
18 weeks	31	119	21
28 weeks	28	117	21
Village STM			
Initial	40	119	26
18 weeks	60	114	20
28 weeks	25	123	22

TABLE III

Serum Cholesterol Levels of Guatemalan School Children
(Effect of Daily Administration of 30 gm. of Lard, Hydrogenated or Plain Cottonseed Oil)

Period	No. of Children	Mean (mg./100 ml.)	Standard Deviation
Group A (SLC)—Lard			
Initial	17	118	17
19 weeks	19	121	17
37 weeks	14	114	18
45 weeks	11	122	16
Group B (SLC)—Hydrogenated Cottonseed Oil			
Initial	27	106	19
15 weeks	17	102	15
26 weeks	13	105	14
Group C (SMC)—Plain Cottonseed Oil			
Initial	103	117	25
18 weeks	105	116	25

COMMENTS

Low serum cholesterol values have been reported in several population groups in the world and have been related to the amount and kind of fat in the diet[7,8] and to a variety of other dietary constituents.[9] The differences in serum cholesterol levels previously observed among different socioeconomic groups in Guatemala are compatible with the hypothesis that they are influenced greatly by the dietary fat. Nevertheless, adding considerable fat to the diets did not raise the serum cholesterol levels of the children in the field trials reported.

Among nondietary influences, racial and familial factors have been considered to influence serum cholesterol levels in man. The studies of Keys et al.[13] and our own findings in rural Costa Rica[2] suggest that in determining the average levels of population groups, these are quite secondary to environmental factors.

Despite evidence suggesting the importance of dietary protein in influencing serum cholesterol levels, Keys and Anderson[14] demonstrated that cholesterol levels in adults did not differ when the diet contained either 10 or 20 per cent of the total calories from skim milk. There are, however, conflicting reports in the literature as to the effect of methionine deficiency. Olsen et al.[15] reported a decrease in serum lipid levels with low methionine-low choline diets in both rats and man and an increase in lipid when either methionine or choline was added to such a diet. On the other hand, Mann and associates[16–18] concluded that methionine, as well as certain other sulfur-containing compounds, exert a specific nonlipotropic effect in lowering serum cholesterol levels in such diverse animals as the rat, chick and monkey. In the present investigation, the administration of 60 gm. daily of skim milk, supplying 522 mg. of additional methionine, did not produce any apparent effect on the serum cholesterol levels of rural school children in Guatemala, even though their habitual diet was relatively low in animal protein. In twenty-four well nourished American men, the administration of 3 gm. of methionine daily for six weeks did not alter the serum cholesterol and lipoprotein levels.[19]

There are many experimental studies, some of them in man, in which the general increase of fat in experimental diets, especially the proportion of nonessential fatty acids, altered serum cholesterol levels. Thus, the failure to demonstrate any serum cholesterol changes when either animal or saturated vegetable fat

was administered to school children consuming a very low fat diet was unexpected. However, the largest part of the habitual diet of the rural Guatemalan child consists of tortillas made from whole corn. It has been observed in animals that corn starch has a lowering effect on serum cholesterol values[9] and also that corn contains a steroid, sitosterol, which also has this effect.[9] Other factors favoring lower serum cholesterol levels are crude fiber and calcium; both are characteristic of a predominantly tortilla diet.[9] All serum cholesterol levels among the groups studied were very low as compared with similar data for the United States or upper income groups in Guatemala.[1–3] It is possible that several different factors in the diets of the rural Guatemalan population act synergistically to maintain low serum cholesterol levels. The supplementary fat was evidently not sufficient to overcome the factors favoring a relative hypocholesterolemia.

While the level of physical activity and possibly also psychological stress are important factors potentially influencing cholesterol levels in the adult population, it is unlikely that children of different socioeconomic groups in Guatemala differ markedly in this regard. We do not believe that these nondietary factors are responsible for the low serum cholesterol levels of rural Guatemalans. On the other hand, we must conclude that manipulating individual dietary factors such as the amount of fat or protein or calories is not enough to affect the balance of all the factors responsible. If indeed these factors determining the low cholesterol levels of rural school children in Guatemala are primarily dietary in nature, a readjustment of the total dietary pattern may be required in order to demonstrate this fact; at least, this is the next field experiment to be tried.

SUMMARY

The administration of 60 gm. of reconstituted dried milk powder daily for twenty-eight weeks had no effect on initial serum cholesterol levels of 101 rural Guatemalan school children. In a related study 30 gm. of fat were given children twice daily in a fried bean and tortilla supplement providing a total of approximately 400 calories. In one (SLC) village, nineteen children received lard (forty-five weeks) and twenty-seven, hydrogenated cottonseed oil (twenty-six weeks); in another (SMC) village all 105 were given cottonseed oil (eighteen weeks). No significant effect on serum cholesterol levels was noted with any of the supplements. Neither frequency of attendance nor gain in weight influenced these results.

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Factors Influencing Serum Cholesterol Levels of Central American Children

II. The Effect of Gross Dietary Changes

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SCHOOL children of low income families in Guatemala have been found to have much lower serum cholesterol levels than those from upper income groups.[1] Nevertheless, in all socioeconomic strata average serum cholesterol levels at birth are closely similar and very low compared to those of school children.[2] Since serum cholesterol differences among socioeconomic groups are marked by the time children have reached school age, it would appear that environmental factors are primarily responsible.

In previous experiments[3] the low serum cholesterol levels of rural children were not influenced by the simple addition of animal protein or of three different types of fat to the habitual diets. In the present study, in order to investigate the effect of more extensive dietary changes on their low serum cholesterol levels, children from families of low socioeconomic status were given diets similar to those consumed among upper socioeconomic groups in Guatemala.

From the Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala. INCAP Publication I-127.

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MATERIAL AND METHODS

Trials were carried out in a small orphanage in Guatemala City (HNP) and in two villages (SMC and MMA) in the highlands of Guatemala. The orphanage children were of either mestizo or Mayan Indian origin while the village children were primarily Mayan Indian. The first trial included, twenty-six children in HNP (twenty-two boys and four girls), averaging eight years of age, 50 pounds of weight and 114 cm. of height. All were given adequate diets which differed primarily in the kind of fat used; during three consecutive four-week periods cottonseed oil, hydrogenated cottonseed oil and lard served in turn as the main source of dietary fat.

The second trial was carried out in the small rural village of SMC in which eighteen children (ten boys and eight girls), averaging six years of age, 36 pounds of weight and 97 cm. of height, were given for a period of eight weeks a diet similar to those of the previous trial. This eight-week study differed in that mixed fat was employed throughout to simulate the fat ordinarily used by high income families.

The third and fourth trials were carried out in the rural village of MMA. In the third trial, twelve children (seven boys and five girls), averaging four years of age, 30 pounds and 90 cm. of height were given the test diet for four weeks. This diet was high in calories from carbohydrate and included a substantial increase in fat intake so that it resembled that of low income urban Guatemalans. In the fourth trial, another twelve children

TABLE I

Diet and Serum Cholesterol Levels in Guatemalan Children in a Poor Orphanage (HNP)

A. *Experimental Diets*

Data	Calories	Protein (gm.)		Carbohydrate (gm.)	Fat (gm.)	Fatty Acids (gm.)*		
		Animal	Vegetable			SAFA	MUFA	PUFA
Initial intake	1,082	9.1	28.8	204.0	15.1	5.8	5.5	2.8
Per cent calories		14.0		75.4	12.6	4.8	4.6	2.3
4 weeks cottonseed oil	2,027	34.5	34.0	356.0	40.6	10.3	11.6	15.1
Per cent calories		13.5		70.3	18.0	4.6	5.2	6.7
4 weeks hydrogenated cottonseed oil	2,043	38.5	32.5	331.0	51.2	13.2	29.2	5.1
Per cent calories		13.9		64.8	22.6	5.8	12.9	2.2
4 weeks lard	1,968	36.8	29.0	298.0	59.4	22.2	25.0	8.2
Per cent calories		13.4		60.6	27.2	10.2	11.4	3.8

B. *Serum Cholesterol Levels*

Data	Initial	4 Weeks Cottonseed Oil	4 Weeks Hydrogenated Cottonseed Oil	4 Weeks Lard
No. of children	24	26	26	24
Serum cholesterol (mg./100 ml.)	119	133	147	152
Standard deviation	22	22	24	26

* In this and the following tables SAFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

(eight boys and four girls), averaging five years of age, 33 pounds and 96 cm. of height, were given a diet for four weeks which contributed calories from fat in proportion similar to that consumed by the upper income urban Guatemalan families.

Throughout all these studies, requirements for minerals and vitamins were adequately met. In each trial dietary surveys were made at the beginning of the experiment and successively during each experimental period by technics previously described.[4] The nutritive value of the diets was calculated using the food composition table prepared by INCAP.[5] For the estimation of the dietary fatty acid intakes, the tables of Hardinge and Crooks[6] and Hayes and Rose[7] were used. All meals were carefully supervised.

Fasting blood samples were obtained by finger tip puncture at the beginning and end of each four-week period in all trials and four weeks after resuming the original diet in trial two. Serum cholesterol was measured by the method of Abell et al.[8] In the analysis of the results the t test for paired comparisons was employed.

DIETARY CHANGES AND SERUM CHOLESTEROL RESULTS

Trial One

Table I shows the daily intake during the trial in group HNP. Total calories, animal and total protein were all greatly increased. The percentage of calories derived from protein, however, was maintained constant throughout the experiment at about 14 per cent. Although carbohydrate intake was increased in amount, the percentage of calories derived from carbohydrate was decreased. Fat intake was greatly increased in net amounts and the resulting percentages of calories derived from fat were 13 per cent in the initial diet, and 18, 23 and 27 per cent, respectively, in the experimental periods. As also shown

TABLE II

Diet and Serum Cholesterol Levels in Guatemalan Children in Rural Village (SMC)

A. *Experimental Diets*

Data	Calories	Protein (gm.)		Carbohydrate (gm.)	Fat (gm.)	Fatty Acids (gm.)		
		Animal	Vegetable			SAFA	MUFA	PUFA
Initial intake	1,032	7.8	22.7	206.0	11.0	2.5	4.1	2.9
Per cent calories		11.8		79.8	9.6	2.2	3.6	2.5
Intake during the study	1,568	39.5	16.8	212.0	57.2	23.1	22.5	7.7
Per cent calories		14.4		54.1	32.8	13.3	12.9	4.4

B. *Serum Cholesterol Levels*

Data	Initial	Treatment		4 Weeks After Resuming Original Diet
		4th Week	8th Week	
No. of children	18	18	18	12
Serum cholesterol (mg./100 ml.)	115	130	129	110
Standard deviation	18	13	18	18

in this table, the saturated, monounsaturated and polyunsaturated fatty acids in the diets were all greatly increased.

The corresponding serum cholesterol results are also shown in Table I. The initial values averaged 119 mg.; after four weeks of the diet containing cottonseed oil, the serum cholesterol levels increased significantly to 133 mg. Changing the source of dietary fat to hydrogenated cottonseed oil caused a highly significant elevation of cholesterol levels to 147 mg. In the last period, when lard was used, an average serum cholesterol level of 152 mg. was observed.

Trial Two

Table II shows the daily dietary intake during the trial carried out in village SMC. Calories, animal and total protein were all greatly increased in absolute amounts. The percentage of calories derived from total protein intake was not significantly increased, and that derived from carbohydrate decreased. The amount of fat was greatly increased and this resulted in an increase from 10 to 33 in the per cent of calories derived from fat. The saturated, monounsaturated and polyunsaturated fatty acids in the diet were all greatly increased.

The resulting serum cholesterol changes during this experiment are also shown in Table II. In the first four-week period, serum cholesterol changed significantly from 115 to 130 mg., but showed no further increase at the end of the eighth week. Four weeks after returning to their habitual diet, the children's serum cholesterol levels returned to 110 mg., a decrease which was significant at the 5 per cent level.

Trials Three and Four

Table III shows the daily dietary intake during the two experiments in village MMA. Caloric intake was greatly increased in trial three and slightly in trial four. Animal protein intake was maintained constant in the third trial and greatly increased in the fourth, although the calories derived from total protein were maintained fairly constant in both. In trial three, carbohydrate intake increased, but the percentage of calories derived from carbohydrate varied only from 78 to 73 per cent. In trial four, carbohydrate intake was reduced in net amount, and percentage of calories from this source lowered from 78 to 54 per cent. Fat was increased in amounts which gave an initial percentage of calories

TABLE III

Diet and Serum Cholesterol Levels in Guatemalan Children in Rural Village (MMA)

A. *Experimental Diets*

Data	Calories	Protein (gm.)		Carbohydrate (gm.)	Fat (gm.)	Fatty Acids (gm.)		
		Animal	Vegetable			SAFA	MUFA	PUFA
Initial intake	1,130	9.0	23.4	221.0	16.6	5.4	7.0	3.4
Per cent calories		11.5		78.2	13.2	4.3	5.6	2.7
First trial	1,736*	9.3	28.6	317.0	37.9	14.2	15.1	5.8
Per cent calories		8.7		73.0	19.6	7.4	7.8	3.0
Second trial	1,197†	24.2	15.8	161.0	44.8	16.2	19.4	5.9
Per cent calories		13.4		53.8	33.7	12.2	14.6	4.4

B. *Serum Cholesterol Levels*

Data	First Trial		Second Trial	
	Initial	Final	Initial	Final
No. of children	12	12	12	12
Serum cholesterol (mg./100 ml.)	146	152	141	142
Standard deviation	24	22	20	20

* Includes estimated house consumption.

† All the meals given at the clinic.

derived from fat of 13.2 per cent and a final value of 19.6 in the third experiment, and 33.7 in the fourth. The saturated, monounsaturated and polyunsaturated fatty acid intakes were increased in both trials.

The resulting serum cholesterol levels during these two studies are also shown in Table III. The values of 146 and 152 mg. per 100 ml. at the beginning and end in the third trial, and 141 and 142 in the fourth, do not represent a significant change. The MMA population differed, however, in that initial serum cholesterol levels were higher than in the previous two trials and were similar to those commonly found among low income families in Guatemala City.[1]

COMMENTS

The influence of dietary changes on serum cholesterol levels of man has been extensively reviewed.[9–11] Although school age children from different socioeconomic groups in Guatemala have strikingly different levels of serum cholesterol,[1] there is evidence that their serum cholesterol levels are difficult to change by dietary manipulation of fat or protein alone.[3]

The present studies show that dietary changes which include not only fat, but other nutrients as well, can elevate the low cholesterol levels of the children of low income Guatemalan families. However, the increase observed in one rural village (SMC), although significant, was only one-third of the difference between low income rural and upper income urban children found in previous studies.[1] Furthermore, in the other village (MMA) in which initial cholesterol levels were already at the level of those of poor urban children, the results were negative.

Although the children under study were of predominantly Mayan Indian origin, their failure to respond to dietary changes cannot be attributed to unique racial characteristics.[12–14] Under given conditions, each subject seems to have a characteristic serum cholesterol level and pattern of variation. In the present trials serum cholesterol concentration changed promptly and noticeably in response to the alterations in diet in some

subjects; in others, the cholesterol levels responded only slowly or not at all.

Many authors have demonstrated that all of the serum cholesterol response as a result of dietary change occurs within the first few weeks.[15–18] A possible exception is a recent abstract by Lee et al.[19] in which they state that Koreans with initially low serum cholesterol and lipid phosphorus levels showed an increase in these levels "within one month" when they were given a high fat diet and that "most of them reached almost as high levels as those of American soldiers of similar age in twelve to eighteen months." In this study serum cholesterol values showed no tendency to increase further beyond the fourth week on an experimental diet. Since the increase observed in four weeks, as a result of the dietary changes, fell far short of compensating for the differences previously encountered between upper and lower socioeconomic groups it appears that other environmental factors, independent of diet as such, must have an important influence on serum cholesterol levels among different socioeconomic groups in Guatemala. These factors are still unknown.

SUMMARY

Twenty-six orphanage children in Guatemala City consuming daily 15 gm. of fat and 38 gm. of protein (36 and 24 per cent animal) were given, during successive four-week periods, adequate diets averaging 37 gm. of animal protein with 41 gm. of fat (18 per cent of calories) predominantly from cottonseed oil, 51 gm. (23 per cent of calories) mainly from hydrogenated cottonseed oil and 59 gm. (27 per cent of calories) primarily from lard. Cholesterol levels increased from 119 (s = 22) to 133 (s = 22), 147 (s = 24) and 152 (s = 26) mg. per 100 ml., respectively.

In a rural Guatemalan highland village eighteen children with cholesterol levels of 115 (s = 18) were consuming daily 11 gm. of fat and 30 gm. of protein (37 and 25 per cent animal). When dietary fat was increased with lard to 57 gm. (33 per cent of calories), and animal protein to 40 gm., cholesterol levels increased significantly to 130 (s = 13) mg. per 100 ml. in four weeks with no further change in eight weeks. Four weeks after resuming the original diet, cholesterol levels were 110 (s = 18) mg. per 100 ml.

It is concluded from these two experiments that dietary changes can elevate the low cholesterol levels of children of low income families, although not sufficiently to account for differences between upper and lower socioeconomic groups in Guatemala. In a similar village in which cholesterol levels of twenty-four children were higher initially (143, s = 29 mg. per 100 ml.) increasing fat with lard from 17 to 45 gm. (34 per cent of calories) with 24 gm. animal protein, did not change cholesterol values. The results of all four trials suggest that factors other than those studied must be important in producing the markedly lower serum cholesterol values in lower as compared with upper socioeconomic groups in Guatemala.

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Infection and Nutritional Status

I. The Effect of Chicken Pox on Nitrogen Metabolism in Children

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THE intense interest in the metabolic effects of severe infections during the early part of the century resulted in studies which clearly established the profound adverse effect of bacterial infections on nitrogen balance. A recent review of available reports on interrelationships between infection and nutrition[1] confirmed the impression that relatively little attention has been paid in recent years to the effect of infection on nutritional status in general and on protein metabolism in particular.

An exception has been the growing recognition that severe protein malnutrition in children, kwashiorkor, is frequently precipitated by an acute infectious episode.[2–8] There are no published studies of the effect of viral infections on nitrogen metabolism, but epidemiologic evidence[9–12] indicates that not only are bacterial infections such as bacillary dysentery important in this regard, but also measles, chicken pox and other diseases of viral origin.

The present study reports the effect of chicken pox on the nitrogen metabolism of children hospitalized in the metabolic unit of the Institute of Nutrition of Central America and Panama (INCAP). Since nitrogen balance studies were already in progress in two of these children, there was an opportunity to determine the metabolic effect of the prodromal as well as the acute phase of the disease.

MATERIAL

Six young boys in different stages of recovery from kwashiorkor were studied. All of them had passed the acute phase and were in the long period of recuperation. Having participated in previous balance studies, the subjects were accustomed to the procedure. Two of them (PC-91 and PC-98) were in a study at the time the first clinical signs of chicken pox appeared. All, except one (PC-98), had relatively severe skin lesions and were febrile during the first three to five days after appearance of the exanthem; the temperature of this patient (PC-98) never exceeded 37.9°c.

In order to obtain complete collections of urine and feces, the children were placed in a bed with a plastic container for the collection of feces protruding through a hole in the mattress, and a funnel of dental plate material was placed around the penis for the collection of urine. Since these were fastened directly to the patient, he could sit up and move about the bed with considerable freedom. Urine and fecal samples were maintained under refrigeration and pooled for each three-day period. Although the frequency of the balance periods varied somewhat from case to case, they generally involved three days on balance and three off. In some cases, observations were continued with diminished frequency of balance periods for three to four weeks after the appearance of the exanthem.

The diet was generally that fed the patient at the time his illness developed, and varied, therefore, from 2 to 5 gm. of protein per kg. of

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TABLE I
Subjects Studied

Subject	Age	Weight (kg.)
PC-82	2 yr.-9 mo.	13.8
PC-91	6 yr.-5 mo.	16.3
PC-92	3 yr.-3 mo.	13.4
PC-97	3 yr.-7 mo.	14.3
PC-98	2 yr.-4 mo.	9.8
PC-102	4 yr.-9 mo.	13.8

body weight per day and 90 to 120 cal. Milk was the source of protein in all the patients but one (PC-98) who received Incaparina, a vegetable mixture of comparable protein quality.[13] All received supplementary carbohydrate and fat as calorie sources as well as adequate vitamins and minerals.

An aliquot of each component of the diet was set aside for analysis, and food not consumed or lost due to vomiting was deducted from the calculated intake. Nitrogen content of food and excreta was determined by the Kjeldahl method and the calculated nitrogen intake was based on actual analysis of the diet aliquots.

The weights and ages of the children appear in Table I.

RESULTS

In the first three cases illustrated in Figure 1, net nitrogen retention decreased in the period immediately following the appearance of the exanthem, although previous balance periods were available in only one (PC-91) of the three to confirm that the drop did not begin at an earlier stage of the disease. In the next two cases (PC-92 and PC-102), the drop in nitrogen retention was delayed until the second balance period after the appearance of the exanthem.

The effect of the infection on three boys (PC-82, PC-91 and PC-97) was of relatively short duration. In two (PC-92 and PC-102) the adverse effect on nitrogen retention persisted for at least two weeks. No systematic differences in the temperature curve or other clinical aspects of these children were noted which might explain the differences in the duration of the effect.

The last case in Figure 1 (PC-98) is more difficult to interpret since a period of low retention was observed five days prior to the appearance of the skin manifestations and again seven days afterwards. It is not certain that these drops in retention were due exclusively to the chicken pox, since the child had only recently recovered from a prolonged period of diarrhea and furunculosis developed soon after the last balance period illustrated. There was, however, no overt evidence of these other disorders during the clinical episode of chicken pox. As noted previously, he remained essentially afebrile throughout, and the skin lesions, although typical, were less numerous than in the other children studied.

Another significant effect of the infection on net nitrogen metabolism (a reduction in intake) is concealed when the data are expressed as percentage of intake. The marked anorexia that developed in association with the infection made it almost impossible to maintain the nitrogen intake of children at predetermined levels, even with the efforts by a trained staff. All the children except one (PC-98), the very mild case, vomited frequently under these circumstances. Vomiting was not limited, however, to the balance periods with reduced retention. As shown in Table II, there is a tendency for nitrogen intake to drop during the acute phase of the disease, although the changes are not of large magnitude in most cases.

The intestinal absorption of nitrogen expressed as percentage of intake and urinary excretion as milligrams nitrogen per kilogram of body weight per day are also shown in Table II. Although the results are somewhat variable, there is no evidence that infection with chicken pox had any consistent effect on absorption. Since the decreased intake of nitrogen was not sufficient to account for the periods of decreased retention, the latter would of necessity have to be the result primarily of increased urinary excretion of nitrogen and the analytical figures for urinary nitrogen in Table II confirm this.

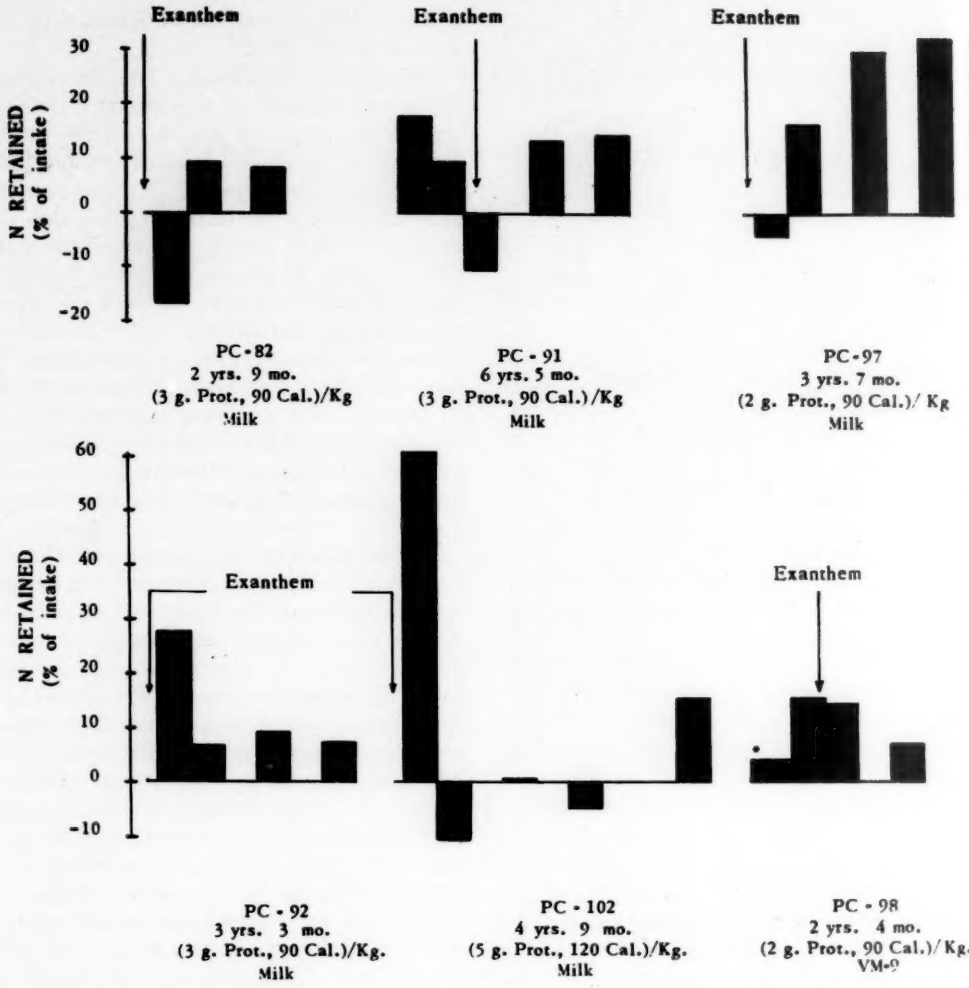

FIG. 1. Effect of chicken pox on nitrogen retention in children (three-day balance periods).

COMMENTS

The results indicate that in each of the six cases studied the disease had an adverse effect on nitrogen metabolism although the timing appears to vary. That the variations observed are not likely to occur in children without an infection is seen in results obtained by us in several hundred other balance periods when infection was not present. In such children the maximum deviations observed over periods of a month or more were much lower than in the present study.

The results also show another common adverse consequence of infection, a reduction of food intake due to the child refusing to consume the same quantity as before the illness developed. Under prevailing practices in areas in which malnutrition is common, this effect could be aggravated by the tendency of parents and physicians alike to reduce the consumption of protein-rich foods during an acute infectious episode.

Another possible effect of infection could be a reduced absorption of nitrogen due to increased gastric motility or interference with biochemical processes involved in absorption at the cellular

TABLE II
Effect of Chicken Pox on Nitrogen Metabolism in Children
(Three-Day Study Periods)

Subject	I	II	III	IV	V	VI	VII	VIII
				Nitrogen Intake (mg. N/kg./day)				
PC-82	↓305	425	NC	419	...	...	...	...
PC-91	452	443	↓392	NC	437	NC	469	...
PC-97	↓251	268	NC	317	NC	242	...	291
PC-92	↓432	363	NC	446	NC	410	...	...
PC-102	↓726	582	NC	685	NC	652	NC*	619
PC-98	341†	338	327	...	309	...	...	...
				Nitrogen Absorption (% of intake)				
PC-82	↓89.4	89.7	NC	90.3	...	...	...	...
PC-91	84.2	82.9	↓78.7	NC	81.9	NC	90.4	...
PC-97	↓71.1	76.9	NC	78.1	NC	80.7	...	...
PC-92	↓87.4	84.3	NC	85.9	NC	83.2	...	...
PC-102	↓80.2	79.6	NC	71.9	NC	70.7	NC*	78.9
PC-98	52.3†	68.7↓	60.9	NC	68.3	...	...	...
				Urinary Nitrogen Excretion (mg. N/kg./day)				
PC-82	↓324	341	NC	344	...	...	...	...
PC-91	302	324	↓349	NC	298	NC	354	...
PC-97	↓188	161	NC	153	NC	146	...	131
PC-92	↓260	281	NC	343	NC	310	...	...
PC-102	↓141	523	NC	484	NC	490	NC*	394
PC-98	164†	181	151	...	189	...	...	...

NOTE: ↓ Exanthem; NC = No collection.
* Seven days.
† Four days.

level in the intestinal wall. There is no evidence from the data presented that this is a factor of importance in chicken pox infections.

The principal effect of the infection seems definitely associated with an increased urinary nitrogen excretion which in turn may be explained by a loss of cell protein due to the infection of individual cells with the virus. An alternate possibility would be a direct interference of the infection with the normal growth and repair of tissue and hence a decrease in protein synthesis. Reiss,[14] on the basis of N^{15} studies, concludes that both impaired synthesis and increased catabolism of protein occurred in the muscles of starved rats with streptococcal pneumonia, with the latter effect predominating.

Infection can also contribute to a lowered nitrogen balance by an increase in basal metabolic rate due to fever. It has been previously shown that the net effect of fever through such a mechanism is small compared to the total magnitude of the change observed, and that large nitrogen losses are often seen in afebrile infections.[15]

All but one of the children in the present study had already recovered from kwashiorkor, and all were receiving an apparently adequate protein intake. In children in lower socioeconomic groups the protein intake is markedly restricted in both quantity and quality, and the effect of an infection on nitrogen metabolism is likely to be more serious, although the absolute change as a result of the infection could be less.[16,17] The data presented help explain why an acute infection in a child who is

already malnourished due to an inadequate protein intake is frequently followed by the development of kwashiorkor.

SUMMARY

Nitrogen balance data were obtained for several three-day periods in each of six children with chicken pox. In all the children some degree of reduction in nitrogen retention was noted. In three of them, this occurred in the period immediately following the appearance of the exanthem, and in two, it was delayed for several more days. In the sixth child, a decrease occurred during the prodromal period. In two of the children the adverse effect lasted for as long as two weeks. Since the infection did not appear to influence nitrogen absorption, an increase in urinary nitrogen was obviously taking place. This was confirmed by direct measurement of urinary nitrogen. Despite efforts to maintain a constant dietary intake, the children consumed less food during the acute phase of infection. The results help explain the association between an epidemic of an acute infection and the subsequent appearance of an increased number of cases of kwashiorkor in children already suffering from underlying protein malnutrition.

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Infection and Nutritional Status

II. Effect of Mild Virus Infection Induced by 17-D Yellow Fever Vaccine on Nitrogen Metabolism in Children

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IT HAS recently been emphasized that a variety of infections, including many of viral origin, may act as stress factors to precipitate the development of kwashiorkor in children already suffering from chronic protein malnutrition.[1–3] While many metabolic studies have demonstrated the marked adverse effect of bacterial infections on nitrogen metabolism in man,[4–6] a recent preliminary report of the effect of chicken pox indicates that viral infections also behave in a similar manner.[7]

The preceding paper in this series[8] reports in more detail the effect of chicken pox on the nitrogen intake, absorption, excretion and retention in six boys of preschool age. Four of these children went into negative nitrogen balance during at least one three-day period following the appearance of the exanthem, and the remaining two children showed a marked drop in nitrogen retention.

There is increasing evidence that many viral infections are of so mild and transient a nature as to escape specific diagnosis.[9,10] When dealing with malnourished populations, the possible metabolic effect of even mild viral infections is of considerable practical significance. The present study was planned to determine whether a viral infection as mild as that induced by immunization in young children with the 17-D strain of yellow fever vaccine would cause a detectable effect on nitrogen balance.

MATERIAL

Nine children, who were patients in a private convalescent home for children in Guatemala City, were selected for study at a time when their recovery from various illnesses was well advanced. Their ages and weights are given in Table I.

Children with intercurrent infections were eliminated from the study by careful clinical examination, although one child (V1) was subsequently found to have a giardia infection which was not treated until the study was completed. No temperature elevation above 38.2°c. was observed in any of the children during either before or after vaccination balance periods even though rectal temperature was measured every four hours.

METHODS

The technics for the collection of urine and feces have been previously described.[8] Intervals of one to three days were allowed between periods in two children (V1 and V2); in the remaining children there was no interval between periods. All children were kept on the basal diet for three days before collections were made. On the first day following four three-day balance periods in two children (V1 and V2), and two periods for the remaining children, 0.5 ml. of yellow fever vaccine containing a neurotropic 17-D virus strain was given subcutaneously.‡ This was followed by

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* This work was carried out during a W. K. Kellogg Foundation fellowship; presently Associate Professor of the Department of Nutrition, School of Public Health, University of São Paulo, Brazil; † Director, INCAP.

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TABLE I
Subjects of Study

Subject	Sex	Age (yr.)	Weight (kg.)
V1	F	8	16.9
V2	M	4	11.7
V3	M	6	18.0
V4	M	8	21.1
V5	M	4	15.1
V7	M	11	21.1
V8	M	4	11.6
V9	M	5	13.4
V10	M	6	12.6

two or four additional three-day balance periods.

The liquid portion of the basal diet was made up of whole milk with added carbohydrate, and the solid part consisted of cooked carrots, bread and bananas. The amounts consumed varied with the child's weight, but the total intake approximated 2.5 gm. of protein and 100 calories/kg. of body weight. All food consumed was weighed and measured and aliquots of the complete diet as well as of the pooled urine and feces for each experimental period were analyzed for nitrogen by the Kjeldahl method. The Student t test was used to evaluate the significance of the differences between mean values.

RESULTS

The average daily intake and the absorption of nitrogen expressed as per cent of total intake are shown in Table II for each individual

TABLE II
Nitrogen Intake and Absorption
After Subcutaneous Injection of the Yellow Fever Virus 17-D Strain
(Three-Day Balance Periods)

Subject	Period					
	I	II	III	IV	V	VI
	Nitrogen Intake (mg. N/kg./day)					
V1	362	473	370*	367†	348	375
V2	524	525	492*	390†	331	384
V3	389	373	386†,‡	405	355§	...‖
V4	371	350	349†,‡	372	350	...
V5	446	475	464†	436	422	394
V7	473	429	421†	419	407	443
V8	439	451	387†	395	474	429
V9	415§	405	377†	350	452	432
V10	432	422	431†	393	446	406
	Nitrogen Absorption (% of intake)					
V1	66.7	62.7	70.6*	60.0†	61.4	59.5
V2	83.6	91.4	83.8†	83.5†	80.6	75.9
V3	77.0	78.2	79.9†,‡	79.1	76.2§	...
V4	77.0	73.2	65.2†,‡	73.3	59.5	...
V5	82.0	74.6	76.5†	79.4	81.0	83.0
V7	82.1	79.9	81.1†	83.5	81.0	81.4
V8	81.4	80.5	84.9†	81.0	85.3	82.7
V9	78.3§	72.0	84.9†	72.6	77.8	78.9
V10	85.0	79.3	81.3†	73.5	81.2	81.0

* Vaccination against small pox attempted immediately prior to this period with no apparent reaction.
† Yellow fever virus 17-D vaccine injected subcutaneously.
‡ The food nitrogen values were taken from the average of the results of the diet of the other balance periods.
§ Analytical error is suspected but could not be located.
‖ Leaders indicate fecal samples lost.

three day period before and after inoculation with the yellow fever vaccine. Although some of the children occasionally vomited part of a feeding, it was generally possible to maintain the planned intake. The changes in absorption of nitrogen following inoculation with the vaccine were random in direction and not significant.

The retention of nitrogen as per cent of intake and the absolute excretion in the urine are given in Table III. In eight of the nine children, nitrogen retention decreased after inoculation and the decrease was significant at the 5 per cent level. In general, a lowered nitrogen retention was observed in all of the periods after inoculation, whether these extended for six or twelve days. Since intake was relatively constant, the decrease in nitrogen retention was due mainly to an increase in nitrogen excretion in the urine; this is apparent from the nitrogen excretion figures given in Table II. When one nonreacting child (V3) was omitted, the increase in excretion amounted to 7 per cent and was significant at the 5 per cent level. On this basis the retention before inoculation averaged 25.3 per cent and after inoculation 16.7 per cent.

COMMENTS

The 17-D strain of yellow fever vaccine was chosen because it was known to be safe and beneficial, and to produce an immune response in a very high percentage of cases. At the same time it could be expected to produce the effects

TABLE III
Nitrogen Excretion and Retention
After Subcutaneous Injection of the Yellow Fever Virus 17-D Strain
(Three-Day Balance Periods)

Subject	Period					
	I	II	III	IV	V	VI
	Nitrogen Excretion (mg. N/kg./day)					
V1	145	142	186*	114†	164	150
V2	334	368	292*	242†	216	235
V3	202	217	218†·‡	229	123§	... ‖
V4	231	216	247†·‡	255	192	...
V5	239	200	259†	241	243	229
V7	209	188	238†	240	213	257
V8	263	262	212†	265	328	292
V9	122§	247	209†	258	310	263
V10	284	240	274†	219	310	300
	Nitrogen Retention (% of intake)					
V1	26.7	32.7	20.4*	29.0†	14.1	19.5
V2	19.8	21.0	24.4*	21.5†	15.4	14.7
V3	24.9	20.2	23.5†·‡	22.6	41.6§	...
V4	14.7	11.5	−5.5†·‡	4.6	4.6	...
V5	28.5	32.6	20.8†	24.1	23.4	23.9
V7	38.0	36.0	24.7†	26.2	28.7	23.4
V8	21.6	22.4	30.2†	13.8	16.1	14.6
V9	48.9§	11.0	29.5†	−1.2	9.2	18.0
V10	19.8	22.4	17.8†	17.8	11.6	14.5

* Vaccination against small pox attempted immediately prior to this period with no apparent reaction.
† Yellow fever virus 17-D vaccine injected subcutaneously.
‡ The food nitrogen values were taken from the average of the results of the diet of the other balance periods.
§ Analytical error is suspected but could not be located.
‖ Leaders indicate fecal samples lost.

of a living virus although its virulence has been depressed by repeated transfers in tissue culture.

In view of the mild and afebrile nature of the reaction to this vaccine, it is somewhat surprising that a consistent decrease in urinary nitrogen excretion was detectable. The positive finding supports the contention of various authors, summarized by Peters and Van Slyke[4] and DuBois,[5] that the effect of infection on nitrogen metabolism is due to an actual destruction of protoplasm, rather than an increased metabolic rate due to fever.

The relatively long duration of the effect of even this very mild viral infection is of considerable potential significance. In an earlier study,[11] a single day of mild diarrhea appeared to be associated with one to two weeks of lowered retention, and in subsequent work with chicken pox and mixed bacterial infections,[8] effects were noted for as long as two to four weeks after the acute illness.

In the present study none of the subjects showing a response attained nitrogen retention values equal to those before inoculation within the six to twelve day period of experiment after inoculation. One (V3) of the nine children failed to show any change in nitrogen retention during the first two periods after inoculation, however in the third period, the nitrogen retention almost doubled. Even assuming that the latter represented an experimental error that could not be detected, there is no suggestion of a negative effect of inoculation in this child. It is possible that for some reason the inoculation did not take; perhaps this child had already acquired some degree of immunity.

If only the results in the eight children showing a response are tabulated, the drop in retention averages 30 per cent of the nitrogen intake and 35 per cent of the nitrogen actually absorbed. Among undernourished children a drop of this magnitude in nitrogen retention could well be of practical significance. Since actual diseases of viral origin, even when they are mild, have even greater adverse effects on nitrogen metabolism, they are likely to contribute significantly to the frequency and severity of protein malnutrition among young children in technically underdeveloped areas. It is hoped that these results will encourage further documentation of the effects of viral diseases not only on nitrogen balance but also on the metabolism of other essential nutrients.

SUMMARY

Eight boys and one girl, ranging from four to eleven years of age, and from 11.7 to 21.1 kg. in weight, were inoculated with yellow fever vaccine. Two to four three-day periods in which nitrogen balance was determined preceded and followed the vaccination. The basal diet contained 2.5 gm. of protein and 100 calories/kg. of body weight. Body temperature was measured every four hours throughout all periods and no fever developed in any of the children. In eight of the nine children, nitrogen retention decreased significantly although neither absorption nor intake were significantly affected. It is possible that the inoculation did not take in the child who showed no change in nitrogen metabolism. In the eight children showing a response, the drop in nitrogen retention averaged 30 per cent of the nitrogen intake and 35 per cent of the nitrogen absorbed. The decreased retention was due almost entirely to increased nitrogen excretion in the urine

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Serum Ascorbic Acid, Riboflavin, Carotene, Vitamin A, Vitamin E and Alkaline Phosphatase Values in Central American School Children

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THE value of determinations of nutrients in blood serum has not been fully appreciated by workers in areas in which the population is well nourished and the range of variation in nutritional status relatively small. In areas in which there is considerable malnutrition, however, blood serum levels of specific nutrients may be useful indicators of nutritional status.[1]

As part of the nutrition surveys of INCAP, it has been possible to determine several serum constituents in persons (principally school children) living in Costa Rica, El Salvador, Guatemala, Honduras and Panama. Serum ascorbic acid, free riboflavin, carotene vitamin A, vitamin E and alkaline phosphatase were measured in over 800 children attending eighteen public schools in both urban and rural areas. To avoid confusing seasonal trends with age trends, seasonal fluctuations in the blood constituents measured were determined from nearly 1,000 adult blood samples. The findings have been useful for comparison with parallel dietary and clinical data from the same populations.

MATERIAL AND METHODS

Blood was taken by venipuncture from children seven to twelve years of age attending public schools in both rural and urban areas of Central America. Blood samples from adults were obtained over a period of two and a half years while the initial nutritional surveys of INCAP were under way. All samples were allowed to clot and then centrifuged within six hours to obtain the serum. Special care was taken to avoid prolonged exposure to light by keeping the tubes containing the blood samples tightly stoppered in a covered portable refrigerator. Aliquots of the serum were taken and stored at approximately minus 20°c., for all determinations except ascorbic acid. For the latter, a trichloroacetic acid protein free serum filtrate was prepared and aliquots of this were stored frozen until the time of analysis. Blood samples from Guatemala were brought directly to the central laboratory, while those from the other countries were sent via air, at convenient intervals, packed in salted ice. All assays were performed within three months after the samples were obtained.

The micromethods of Lowry et al.[2] for the determination of serum ascorbic acid, Burch et al.[3] for riboflavin, Bessey et al.[4] for vitamin A and Bessey et al.[5] for alkaline phosphatase were used. At the time these determinations were made, the measurements of "free" riboflavin were not corrected for the contribution of flavin-adenine dinucleotide riboflavin. Vitamin E was determined according to Quaife et al.[6] while carotene was estimated inter-

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TABLE I
Blood Serum Values in Central American School Children

No. of Children	Constituent of Blood Serum	Mean	Standard Deviation
850	Free riboflavin (μg./100 ml.)	1.53	0.93
850	Ascorbic acid (mg./100 ml.)	1.55	0.61
849	Vitamin A (μg./100 ml.)	26.4	8.4
849	Carotene (μg./100 ml.)	90.0	44.0
692	Vitamin E (mg./100 ml.)	0.65	0.26
868	Alkaline phosphatase (mM/L./hr.)*	5.25	1.99

* Milimoles of p-nitrophenyl phosphate per liter per hour. Approximately equal to 1.79 Bodansky units.

changeably by the method of Bessey et al.,[4] or the method of Quaife et al.[6] The results obtained from these two procedures proved identical for practical purposes and the simultaneous determination of total carotenoids with either vitamin A or vitamin E simplified the laboratory routine.

RESULTS

The average free riboflavin, vitamin C, vitamin A, carotene, vitamin E and alkaline phosphatase serum values for school children in Central America, with their standard deviation, are given in Table I. These values were obtained from the total sample which includes children from both urban and rural lower income populations. Mean values for these serum nutrients, according to country and place of residence, are given in Table II. School children from both urban and rural communities were studied in Guatemala and El Salvador. Children living in rural areas had lower serum values for vitamin A, carotene, vitamin E and alkaline phosphatase, but higher serum values for riboflavin than children living in urban communities. Serum levels of ascorbic acid were high in the urban Guatemalans and much higher in the rural school children from El Salvador.

The seasonal variations in nutrient levels in the adult group sampled are shown in Figure 1. The levels of all the nutrients except riboflavin tended to be highest from November to March, which are dry months, and lowest during the rainy season months, May to September. Riboflavin behaved in the reverse manner with a peak in July. The average levels for ascorbic acid tended to be lower in the adult group than in school children in the same area, but the levels for vitamin E, carotene, vitamin A, and free riboflavin were unaltered or higher. Seasonal trends for alkaline phosphatase were not evaluated because of insufficient determinations in adult blood samples for this purpose.

TABLE II
Blood Serum Values in Central American School Children by Country and Place of Residence

Country	Place of Residence	Maximum No.*	Free Riboflavin (μg./100 ml.)		Ascorbic Acid (mg./100 ml.)		Vitamin A (μg./100 ml.)		Carotene (μg./100 ml.)		Vitamin E (mg./100 ml.)		Alkaline Phosphatase (mM/L./hr.)†	
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Costa Rica...	Urban	145	1.45	0.94	1.47	0.42	28.4	10.2	75	37	0.74	0.22	4.65	1.40
	Urban	131	1.65	0.96	1.28	0.45	25.8	8.1	85	35	0.80	0.26	4.96	1.68
El Salvador..	Rural	86	1.44	0.70	2.65	0.87	19.6	7.7	44	32	0.55	0.20	4.88	1.52
	Urban	19	1.34	0.72	1.85	0.24	30.5	10.1	157	60	0.44	0.20	6.98	1.80
Guatemala...	Rural	232	1.66	1.15	1.14	0.42	24.8	7.1	108	50	0.55	0.24	5.22	2.06
Honduras....	Urban	92	1.73	0.99	2.66	1.14	28.6	9.6	57	28	0.67	0.20	5.49	1.44
Panama.....	Urban	183	1.32	0.71	1.37	0.39	25.0	14.3	101	60	...	...	6.19	3.20

* Minor differences in number of determinations occurred for the different constituents because of breakage or loss.
† mM of p-nitrophenyl phosphate per liter per hour. Approximately equal to 1.79 Bodansky units.

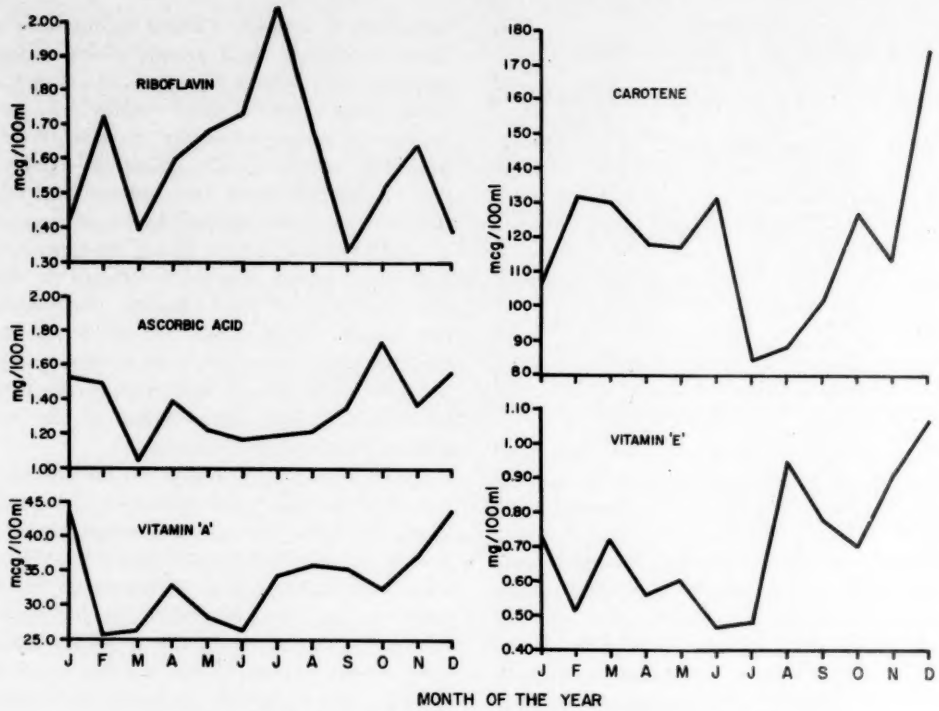

FIG. 1. Seasonal trends in serum values in Central American adults.

COMMENTS

Serum protein levels for the Central American area have been previously described.[7] The data indicate that not only are all of the average values well within normal limits, but also very few individual values fall below the 6 gm. level which has been considered to be critical from a public health point of view.[1,8] Persons consuming diets in which the protein is predominantly of vegetable origin are known to have serum protein values the same or even higher than those whose diets contain abundant animal protein.[7] Thus, serum protein values reveal protein malnutrition only when it is sufficiently severe as to be manifest by clinical signs. No child attending school was found to have reached this degree of protein malnutrition, although cases of kwashiorkor are common in most of the populations studied.[9,10]

The average values reported for serum ascorbic acid, riboflavin, vitamin A, carotene, vitamin E and alkaline phosphatase were well above levels considered to represent deficiency.[1,11–17] Since the standard deviations are large, however, levels suggestive of deficiencies for some of the nutrients were found in many children.

Average values for riboflavin in school children were quite uniform among the groups sampled and also within normal limits[13] although the estimated dietary intake is approximately 50 per cent[18,19] of the recommended INCAP[20] and N.R.C.[21] allowances. In physical examinations among these children, signs suggestive of riboflavin deficiency, such as cheilitis, angular stomatitis and nasolabial seborrhea, were found only rarely, although hypertrophy or atrophy of the papillae of the tongue are regularly found in about 15 per cent of the children.[22]

The levels of ascorbic acid are considerably higher than those reported for school children in the United States[23] where a level of 1 mg. per 100 ml. has been considered an indication of saturation associated with dietary intakes of 75 to 100 mg. per day.[24,25] Even though

average values in excess of 2.6 mg. per 100 ml. were encountered in the population of urban Honduras and rural El Salvador, and no group averaged below 1.1 mg. per 100 ml., dietary surveys in the area did not estimate dietary intakes in excess of 50 mg. per day. Since analytical error has been ruled out by repeated analysis of known serum samples, it seems probable that although the diets are exceedingly rich in ascorbic acid, much fruit, which is the major source of ascorbic acid, is consumed between meals and not reported to the nutritionists as part of the food consumption measured in dietary surveys. The same type of difficulty has been encountered in other tropical regions in measuring the relatively large amount of fruit consumed between meals.[26]

According to dietary surveys, vitamin A is probably the most deficient vitamin in the diet in all the countries represented in the study. Vitamin A activity of the diets is estimated to vary from 690 μg. (2,300 I.U.) in localities where green leaves are more abundant and used in the regular diet[18] to 290 μg. (980 I.U.) in communities where the diet is relatively lacking in both animal and vegetable sources of vitamin A activity.*

In the clinical examinations, frank xerophthalmia and Bitot's spots are not found in school children, and such common suggestive signs as mild xerosis and follicular hyperkeratosis are notoriously nonspecific. The mean values for vitamin A and carotene are both considerably lower than those for comparable groups in the United States, and individual values below the 15 to 20 μg. per 100 ml., which Bessey[17] considered to be suggestive of deficiency, were observed in about 15 per cent of the children.

Carotene levels were highest in the population of Guatemala, especially in the highland areas, where green leaves and yellow corn are consumed in larger quantities than in the other countries. It is probable, however, that some of the carotene activity measured represents inactive carotenoid pigments. The carotene levels are relatively low in the other countries and suggest that the intake of biologically active carotenoids is probably inadequate to make up for the relative lack of preformed vitamin A in the diets of children in the lower income groups.

Little is known about either the normal serum level of vitamin E or its significance in human nutrition, although it has been established that the vitamin E level in plasma raises and falls with intake in both experimental animals and man.[17] The large whole grain cereal consumption of populations in the lower income groups in Central America should ensure an abundance of this vitamin in the diet. All the average values in the present study were within the limits of values for population groups considered well nourished.[15,16]

The alkaline phosphatase values for Central American school children are below those reported for well nourished children of the same age in the United States. No elevated alkaline phosphatase values suggestive of rickets were encountered. A possible interpretation of the lowered alkaline phosphatase values is that they reflect a poor nutritional status with respect to protein or other nutrients. This is supported by the finding that alkaline phosphatase values in Guatemalan and foreign children from upper income families attending a private school in Guatemala City, are higher than those in Guatemalan children from low income groups and similar to those found in the United States.[27] In severe protein malnutrition (kwashiorkor), markedly lowered alkaline phosphatase is one of the biochemical characteristics of the syndrome.[28,29]

The apparent seasonal trends in the serum values of all the nutrients studied are of particular interest because they suggest that the levels are sensitive to seasonal variations in nutrient intake. Fruits rich in ascorbic acid are abundant at all times; their price is lower, however, and certain foods which are particularly good sources such as mangoes (Mangifera indica L.), guavas (Psidium guajava L.) and jocotes (Spondias purpurea L.) are more available during the dry season. Many of these same fruits are also sources of biologically

* INCAP unpublished data, kindly supplied by Miss Marina Flores.

active carotene and would be partly responsible for the variations in serum carotene and vitamin A.

SUMMARY

Microchemical analyses of blood samples taken from school children in different surveys conducted in Central America give the averages for serum constituents shown in Table I.

Comparison of the present results with similar studies in the United States indicates that in the Central American children studied, the serum ascorbic acid values were higher, the serum riboflavin and vitamin E values were in the same range, and the serum vitamin A and total carotenoids were lower. No elevated serum alkaline phosphatase values were encountered. In approximately 1,000 adults, ascorbic acid, vitamin A, total carotenoids and vitamin E levels were highest during the dry season, while riboflavin values tended to be highest during the rainy season.

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Urinary Excretion of Creatinine of Children under Different Nutritional Conditions

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IN 1908, Shaffer[1] demonstrated that the daily excretion of creatinine in adult man is remarkably constant over long periods of time, even under the most varied circumstances. Because the quantity excreted is little affected by diet, exercise or volume of urine, the daily creatinine excretion is used to determine the completeness of twenty-four hour urine collections.

Since creatinine excretion is determined principally by lean body mass,[1-3] the number of milligrams excreted in twenty-four hours per kilogram of body weight, the "creatinine coefficient," has long been used as an index of the relative amount of muscle in an individual. The daily creatinine excretion of obese persons is low in relation to their ideal weight.[5] When overweight is reduced by dietary means, the absolute excretion of creatinine does not change. On the other hand, persons with below normal weights tend to have abnormally low "creatinine coefficients" which reflect subnormal development of muscle mass.

Recently, Stearns et al.[6] have summarized the results of twenty-five years of investigation in a "creatinine coefficient" curve for well nourished children one to ten years of age in Iowa. Both Talbot[7] and Daniels and Hejinian[8] have used the "creatinine coefficient" as an index of nutritional status in children although the latter prefer to relate creatinine excretion to height for this purpose.

We have adopted the expression *milligrams of urinary creatinine per centimeter of body height;* since it is not affected by the amount of adipose tissue, it is a better indicator of the relative development of lean body mass than the commonly employed "creatinine coefficient." The present study presents preliminary observations on the excretion of creatinine per centimeter of height in children with nutritional status varying from excellent to acute kwashiorkor.

MATERIAL

Group A

Urban Upper Socioeconomic Group. Fifteen students of a private school in Guatemala City, who ranged in age from six years five months to eight years seven months, were studied together with eight children, who ranged in age from two years two months to five years five months, from families of local professionals.

Group B

Rural Lower Socioeconomic Group. Eight children living in the rural highland village of Santa María Cauqué were studied; they ranged in age from three to six years.

Group C

Patients with Kwashiorkor. This group included twenty-two patients hospitalized with acute kwashiorkor in the metabolic unit of INCAP. The urinary excretion of creatinine was measured in nine of these children during the first days of hospitalization and again six to eighteen months after the child was considered to be clinically recovered. Only initial values were obtained in nine cases and values only after recovery in the remaining four.

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TABLE 1
Daily Intake of Preschool Children as Percentage of INCAP Recommended Allowances
(Eight Cases per Group)

Nutrient	INCAP Recommendation	Socioeconomic Level			
		Urban Upper		Rural Lower	
		%	Range	%	Range
Calories	1,425	133	112–156	73	36–111
Total protein (gm.)	47.5	145	106–171	62	26–97
Animal protein (gm.)	15.8	332	204–422	50	0–139
Riboflavin (mg.)	1.15	268	184–364	37	12–55
Niacin (mg.)	7.12	146	123–184	77	48–105
Thiamin (mg.)	0.75	176	128–318	86	44–126
Ascorbic acid (mg.)	46.00	240	144–524	43	16–110
Vitamin A (I.U.)	2,375	413	116–1,150	47	3–121

METHODS

The urine was collected during twenty-four hours, except in the fifteen students of the private school, in whom only three-hour collections were made. When twenty-four-hour collections were not made, the twenty-four-hour excretion of creatinine was obtained by calculation. High correlations have been found in our laboratory between "muscularity" and twenty-four-hour urinary creatinine excretion values per centimeter of body height calculated from three-hour specimens from twelve boys and fifteen girls. The former was obtained from arm circumference and skinfold data using the formula of Brozek.[9] The correlation coefficients were 0.96 for boys ($P < 0.001$) and 0.85 for girls ($P < 0.05$). Further evidence of the feasibility of using short collection periods has been obtained from a study in twelve men and seven women in whom twenty-four-hour excretions calculated from three-hour collections (men, 1,644 mg.; women, 962 mg.) were not significantly different from those in twenty-four-hour specimens (men, 1,627 mg.; women, 964 mg.).

In the patients with kwashiorkor temporary retention of creatinine, probably of renal origin, was observed initially. This impairment disappears during the first few days of treatment with a resulting sudden rise in urinary creatinine followed by a decrease.[10] The sudden initial rise could not be due to a gain in muscle tissue since it is not conceivable that protein mass could increase so rapidly. We have called this lower more stable level the "initial" excretion of creatinine.

Toluene was used as a preservative, and the samples stored at minus 20°c. until they could be analyzed. The creatinine determinations were made by the method of Clark and Thompson.[11] The heights of all the children were obtained at the time of collection. In eight children in each of groups A and B, the food intake was estimated by means of personal interviews with the mothers.*

RESULTS

Dietary Characteristics

Table 1 presents the estimates of the intake of nutrients expressed as percentages of INCAP's dietary recommendations for children of the average age of the group studied.[12] These differ from those of National Research Council (N.R.C.) only in lower levels for calcium and vitamin C. The contrast between the relatively low intake of the rural group and the relatively excessive consumption of many nutrients of the children of the upper socioeconomic group should be noted.

The dietary intake of the children with kwashiorkor immediately before hospitalization is extremely variable and can only be

* The collaboration of Miss Marina Flores, Section Chief of Dietary Surveys, and Berta García and Zoila Flores, is appreciated.

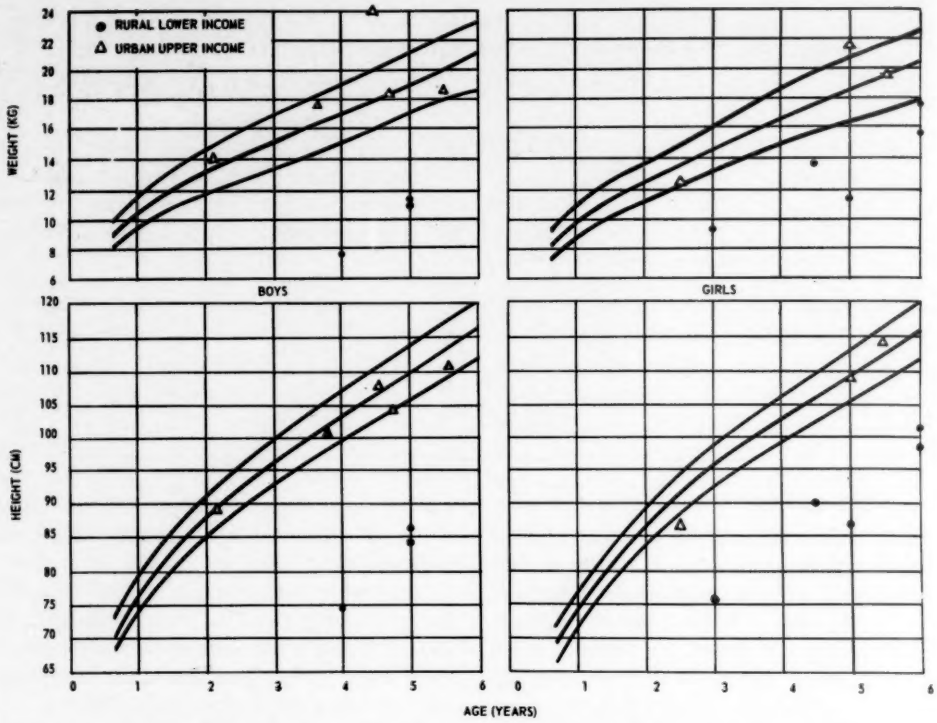

FIG. 1. Body heights and weights of preschool children of two different socioeconomic groups in Guatemala. In this figure and in Figure 2, for comparison purposes, INCAP standards for height and weight for Central America are reproduced in solid lines. The solid middle line represents the median for weight and the mean for height. The upper and lower solid lines represent the 84th and 16th percentile for weight and plus-minus 1 standard deviation for height.

estimated approximately from the history given by the mother upon the child's admission to the hospital. However, these children's diets are even poorer than those ordinarily consumed by children of the socioeconomic group from which they come. Furthermore, due to anorexia and erroneous dietary practices, the protein intake is restricted even more when the first signs of the disease become manifest.[13]

Although a direct intake of food was not investigated in the school children of the urban area, a previous study of mothers of children belonging to this group revealed that their diets contained adequate amounts of all essential nutrients.

Physical Measurements

Figures 1 and 2 confirm that children of the lower rural socioeconomic group and the patients hospitalized with kwashiorkor are markedly reduced in height and weight compared with those of high socioeconomic group.

Biochemical Measurements

In Figure 3 are illustrated the creatinine excretions, in milligrams per centimeter of height, of the different groups of children studied. The average values and their standard deviations found by Stearns[15] in adequately nourished North American children, have been included for the purpose of comparison. Seven individual values for infants from four to seven months of age, given by Daniels and Hejinian,[8] have been included. The values found for adequately nourished children in the present study generally agree with those in the papers cited.

TABLE II
Urinary Creatinine Excretion per Centimeter of Body Height in Children with Kwashiorkor

Subject	Initial		Recovered	
	Age	Creatinine (mg./cm./24 hr.)	Age	Creatinine (mg./cm./24 hr.)
PC-67	1 yr. 3 mo.	0.8	1 yr. 9 mo.	1.2
PC-82	1 yr. 4 mo.	1.0	3 yr.	1.8
PC-66	1 yr. 7 mo.	1.0	2 yr. 7 mo.	1.3
PC-83	1 yr. 10 mo.	0.9	2 yr. 7 mo.	1.6
PC-98	1 yr. 11 mo.	1.1	2 yr. 7 mo.	1.5
PC-86	2 yr. 8 mo.	1.1	3 yr. 7 mo.	2.0
PC-95	2 yr. 9 mo.	1.1	3 yr. 3 mo.	2.1
PC-102	4 yr. 9 mo.	2.0	5 yr. 3 mo.	2.5
PC-101	5 yr. 2 mo.	1.4	5 yr. 9 mo.	2.2

The children of the rural lower socioeconomic group excreted much smaller quantities of creatinine. Although the group of children with kwashiorkor were in general younger, their excretion fell on the line projected from data obtained from the rural lower socioeconomic group.

The levels of excretion in the children recovered from kwashiorkor were considerably higher. These increases with recovery are more clearly illustrated in Table II which lists those cases in which the determination was made during both the acute phase and several months after clinical recovery. In none of these children did creatinine excretion reach normal values even though they received good diets for periods ranging up to eighteen months.

COMMENTS

The amount of creatinine excreted by an individual is recognized as a good estimate of the relative amount of skeletal muscle. Exceptions to this are found under certain pathologic conditions, such as renal diseases and excessive muscle wastage, which are easy to recognize clinically and uncommon. In their work on the protein requirements of children one to ten years of age, Stearns et al.[6] suggest that the protein intake which permits the formation of an amount of muscle characteristic for a specific age group, should be considered the protein requirement for that age.

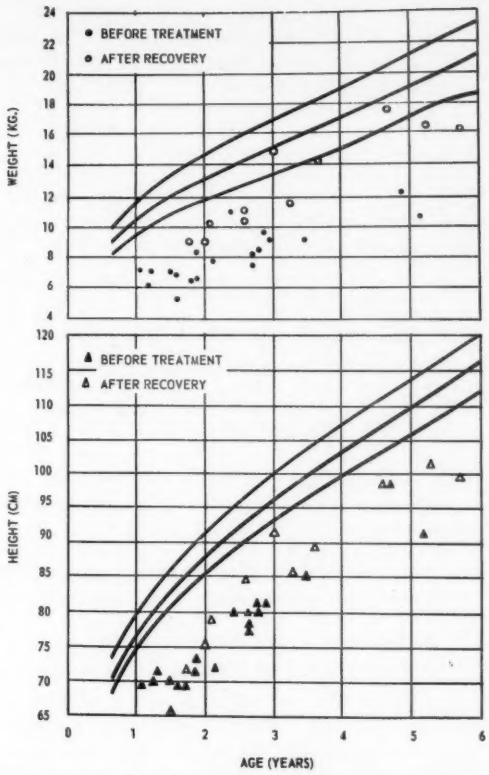

FIG. 2. Body heights and weights of children with acute kwashiorkor and after clinical recovery.

It is known that considerable skeletal muscle may be sacrificed during periods of protein deprivation to prevent the loss of protein from more important tissues. It would seem, therefore, that the protein requirements of tissues of high biological priority have been satisfied if the amount of skeletal muscle has been well maintained.

Our finding that the figures for creatinine excretion per centimeter of height in adequately nourished Guatemalan children coincide with those found by Stearns in North American children, indicates that the relative amount of muscle is the same in both groups. On the other hand, the excretion values for children of the rural lower socioeconomic group, three to six years of age, closely approach the values given by Daniels and Hejin-

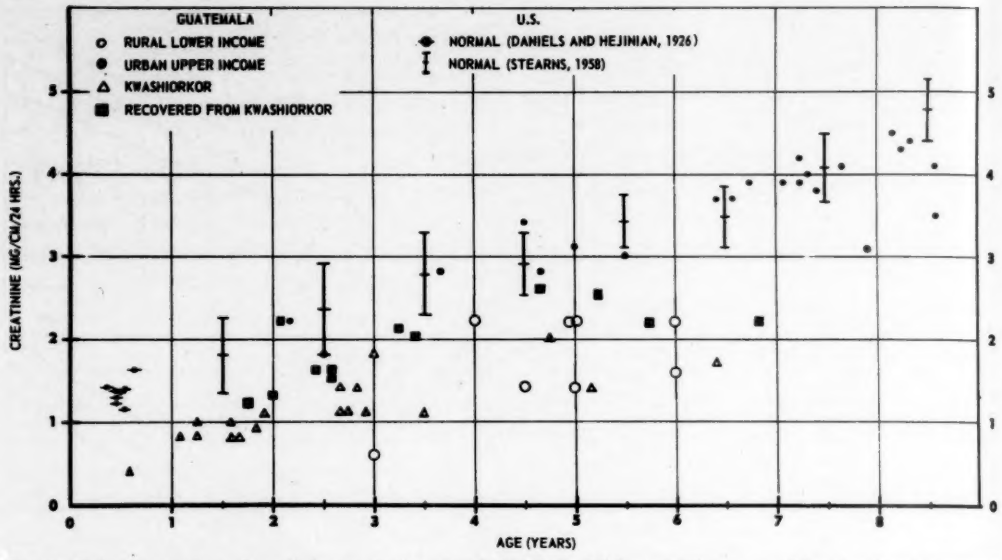

FIG. 3. Urinary excretion of creatinine in relation to body height in children of different nutritional and socioeconomic status.

ian for children four to seven months of age.[8] These values are also similar to those found in patients with kwashiorkor upon admission to the hospital. Thus, apparently healthy preschool children belonging to the economical and nutritionally underprivileged groups in Guatemala do not seem to differ radically from those with kwashiorkor as far as the magnitude of the protein depletion is concerned. The acute picture of kwashiorkor may be due to superimposed factors acting on undernourished children who are, in terms of creatinine excretion at least, ambulatory cases of latent kwashiorkor.

Although the children with kwashiorkor markedly increased their ratio of creatinine: height during recovery, reflecting a rapid muscular repletion, the majority did not reach the levels found in adequately nourished children of the same age, even after six to eighteen months of treatment. This suggests that the retardation in development resulting from the chronic severe protein deficiency suffered by these children is not readily overcome.

SUMMARY

The twenty-four–hour urinary excretion of creatinine per centimeter of body height was studied in three groups of children in Guatemala. Twenty-three came from an urban upper socioeconomic group and were adequately nourished; eight were from a rural lower socioeconomic group and twenty-two were patients with kwashiorkor.

The data from the adequately nourished children coincided with those for healthy North American children, indicating their relative muscle mass to be similar. The children from the rural group and those with kwashiorkor excreted much less creatinine per centimeter of body height. The results indicate that in Guatemala, economically and nutritionally underprivileged children without overt malnutrition do not differ radically in the magnitude of protein depletion, as indicated by creatinine excretion, from those with kwashiorkor. With recovery from kwashiorkor, a marked increase was observed in the creatinine excretion of the patients with kwashiorkor, but in the majority levels normal for their age were not reached even after six to eighteen months of treatment.

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Serum and Urinary Creatinine in Children with Severe Protein Malnutrition

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IN Central America[1] as in Jamaica[2] and Africa,[3] children suffering from the severe protein deficiency of kwashiorkor have been found to have a very low urinary output of creatinine. Standard et al.[2] found that during recovery urinary creatinine increases proportionally to the increase in muscle mass. However, about half of their patients and most of ours showed an abrupt transient rise in creatinine excretion during the first few days of treatment, followed by a variable drop, a phenomenon which seemed more marked in children with edema. This suggests that urinary excretion of creatinine in the acute phase of kwashiorkor may be temporarily reduced by factors other than decreased muscle mass. In this case urinary excretion of creatinine could not properly be used to estimate relative muscle mass of children with severe protein malnutrition. If children with kwashiorkor can be shown to have a reduced renal capacity to eliminate endogenous creatinine, this would explain the marked transient rise frequently observed when treatment is initiated. The present study measures the urinary excretion of creatinine and the serum creatinine concentration of children during the acute phase of kwashiorkor and at intervals during recovery. Creatinine clearances were also calculated and compared with data obtained from clinically healthy adequately nourished children.

From the Institute of Nutrition of Central America and Panama (INCAP) Guatemala. INCAP Publication I-151.

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MATERIAL

Children with Kwashiorkor

Nine children hospitalized with kwashiorkor were studied. Their initial age, total serum proteins, body surface and twenty-four-hour urine volume are included in Table I. All had severe hypoproteinemia and edema, although in one child (PC-95) the edema was mild. Severe oliguria was present in one child (PC-100), and to a lesser degree in two others (PC-102 and PC-103). With adequate protein therapy all showed satisfactory initial recovery, during the period of observation. The initial decrease in body surface seen in all but one case (PC-67), was due to the disappearance of edema. In the five children in whom the observation period was prolonged beyond the initial few days, the characteristic return of the total serum proteins to normal levels occurred.

Well Nourished Control Children

Seventeen children were selected from a private school. They belonged to the upper socioeconomic group of Guatemala City, and according to physical measurements and clinical examination were well developed and in good health. Their age, total serum proteins, body surface and three-hour urine volumes are included in Table II.

METHODS

The children with kwashiorkor were placed in a bed specially adapted for complete urine collection, while the control children voided

TABLE I
Urine and Serum Creatinine Changes in Children Recovering from Kwashiorkor

Subject	Age	Days from Admission	Body Surface ($M.^2$)	Serum Protein (gm./100 ml.)	Hematocrit (%)	Urine Volume (ml./24 hr.)	Creatinine		
							Urine (mg./24 hr.)	Serum (mg./100 ml.)	Clearance (ml./min./M^2)
PC-82	1 yr. 3 mo.	0	0.44	3.92	27	424	54	0.68	13
		7	0.40	5.09	28	618	80	0.54	26
PC-66	1 yr. 6 mo.	0	0.41	3.64	31	683	49	0.76	11
		12	0.40	4.68	31	536	66	0.49	24
PC-67	1 yr. 6 mo.	0	0.37	4.69	27	555	51	0.44	23
		8	0.37	4.75	31	694	69	0.38	29
PC-98	2 yr.	0	0.45	3.29	29	301	68	0.79	13
		8	0.41	4.42	26	101	78	0.72	18
		35	0.43	7.02	..	590	120	0.55	35
		105	0.48	6.65	..	336	118	0.46	38
PC-103	2 yr. 8 mo.	0	0.45	3.96	35	112	64	0.75	13
		5	0.44	4.00	31	123	86	0.54	25
		21	0.44	6.90	..	703	56	0.56	16
		63	0.51	7.33	..	918	148	0.57	35
PC-95	2 yr. 9 mo.	0	0.47	3.57	30	182	66	0.88	11
		12	0.44	4.77	24	863	89	0.47	30
		35	0.47	5.81	..	344	84	0.47	33
		180	0.56	7.40	..	700	181	0.49	35
PC-100	3 yr. 10 mo.	0	0.51	2.83	37	36	45	0.91	7
		6	0.48	3.70	30	44	67	0.31	31
PC-102	4 yr. 8 mo.	0	0.63	3.13	34	76	144	1.07	15
		13	0.59	5.45	34	850	195	0.72	32
		30	0.64	6.41	..	367	246	0.52	51
PC-101	5 yr.	0	0.57	3.05	36	405	111	0.80	17
		20	0.54	4.40	37	625	125	0.65	25
		45	0.57	6.33	..	845	154	0.57	33
		90	0.63	6.28	..	586	158	0.48	36

voluntarily. The urine was preserved with toluene and stored at minus 20°c. until analyzed. Blood was obtained by pricking the fingertip or by venipuncture sometime during the urine collection period, the serum separated and kept at minus 20°c. Creatinine was determined by the method of Clark and Thompson[4] in both the urine samples and in tungstic acid filtrates of the serums. Total serum proteins were determined by the density gradient method of Lowry and Hunter.[5] Body surface area was calculated from height and weight data employing the equation of Boyd and Scammon.[6] Clearances were calculated by the equation $U \times V/S$ in which U and S are the concentrations of creatinine in urine and serum per 100 ml., respectively, and V is the volume of urine per minute. The collection periods were as close as possible to three hours for the control children and twenty-four hours for the patients with kwashiorkor.

RESULTS

The data in Table I indicate a rapid rise in the twenty-four-hour urinary excretion of creatinine and a simultaneous fall in serum creatinine concentration during initial recovery from kwashiorkor. With the exception of one child (PC-98), the fall cannot be accounted for by hemodilution as estimated from changes in hematocrit. Also shown in Table I are the renal clearances expressed per square meter of body surface, which were markedly reduced on admission to the hospital and which increased rapidly during the first few days of therapy.

Renal clearance values obtained in five of the patients with kwashiorkor after at least

TABLE II
Urine and Serum Creatinine in Healthy Children

Child	Age	Total Serum Protein (gm./100 ml.)	Body Surface (M^2)	Urine Volume (ml./3 hr.)	Creatinine		
					Urine (mg./3 hr.)	Serum (mg./100 ml.)	Clearance (ml./min./M^2)
R. R.	6 yr. 5 mo.	6.65	0.77	105	45	0.83	42
J. H.	6 yr. 7 mo.	6.40	0.88	115	51	0.62	56
J. W.	6 yr. 9 mo.	6.51	0.83	90	49	0.83	43
A. S.	7 yr. 1 mo.	6.40	1.07	57	66	0.88	37
B. E.	7 yr. 3 mo.	7.25	0.87	135	59	0.73	50
F. N.	7 yr. 3 mo.	6.75	1.17	218	71	0.76	44
H. A.	7 yr. 4 mo.	6.50	0.84	69	60	0.81	50
G. A.	7 yr. 5 mo.	6.65	0.94	30	41	0.69	33
A. L.	7 yr. 5 mo.	7.10	0.83	91	54	0.81	47
L. M.	7 yr. 8 mo.	6.65	0.95	143	66	0.74	51
A. F.	7 yr. 11 mo.	6.50	0.83	47	43	0.86	35
J. C.	8 yr. 2 mo.	6.75	1.03	101	73	0.91	44
L. Mo.	8 yr. 3 mo.	6.70	0.90	79	66	0.79	51
F. P.	8 yr. 4 mo.	6.52	1.03	48	77	0.88	43
Jo. W.	8 yr. 7 mo.	6.52	0.94	125	67	0.84	47
S. O.	8 yr. 7 mo.	7.25	0.88	34	55	0.79	45

thirty days of therapy are given in Table I and those for clinically healthy, well nourished children are shown in Table II. These data indicate that as early as one month after initiation of treatment, the children recovering from kwashiorkor reach clearance values similar to those of the control children. The serum creatinine concentration of the children with kwashiorkor, not only decreased during the first few days of therapy, but also remained markedly lower than the serum creatinine levels found in the control children.

COMMENTS

It is not conceivable that the lean body mass could increase so markedly in so short a time as to explain the rapid rise in the urinary excretion of creatinine. Furthermore, the parallel decrease in the serum creatinine levels, indicates a relative initial retention of creatinine. It is striking that the impairment in creatinine excretion in the patients studied was so readily reversed during initial recovery.

The fact that in adults, the endogenous creatinine clearances and inulin clearances are equivalent,[7] is evidence that creatinine is excreted by glomerular filtration and that tubular reabsorption does not occur. In the present study, creatinine clearance values obtained by us in normal children averaged 44 ml. per minute/M^2. This is 64 per cent of the clearance reported by Sirota et al.[8] of 69 ml. per minute/M^2 for the adult subject, and 68 per cent of the average values found by us for eighteen clinically healthy male adults twenty to thirty-five years of age (65 ml./minute/M^2).[9] Broad and Sirota[7] studied four infants, two months to twenty-five months of age, and found (in agreement with our results) that their endogenous creatinine clearance was only about 60 per cent of the inulin clearance. These authors speculated that in infants the "picric acid-chromogenic material" measured by the method employed, is excreted by the kidney in a different manner than in adults, an explanation which could well apply to our findings for normal children. Nevertheless, the extremely low clearances found in children with acute kwashiorkor indicate an even more reduced renal excretion of creatinine which could be due to either tubular reabsorption, decreased glomerular filtration rate or reduced renal plasma flow. Although the normal tubules do not reabsorb endogenous creatinine, such a mechanism might come into play in the protein-depleted child. On the other hand, functional impairment of the glomeruli and reduction in renal plasma flow

are suggested by the findings of Gordillo et al.[10] that the clearance of inulin as well as that of para-amino hippuric acid are decreased in severe chronic infantile malnutrition.

Standard et al.[2] have pointed out the association between the transient initial changes in creatinine excretion in children with kwashiorkor and the loss of edema. In the present investigation all the children studied had edema on admission to the hospital, and loss of edema invariably accompanied the changes in serum and urinary creatinine. It is possible that the renal impairment which is causing the retention of creatinine is also contributing to the formation of edema. Parallel studies of severely malnourished children without edema, i.e., marasmus, might be helpful in evaluating this possibility.

No explanation has been found for the observed persistence of very low serum creatinine concentrations in children after recovery; the duration of this effect and the severity of protein malnutrition necessary to produce it are under investigation in our laboratory. The immediate practical implication of the results described is that the values for creatinine excretion obtained in children with acute kwashiorkor are not valid for comparative estimations of the increase in muscle mass during early recovery.

SUMMARY

Nine children hospitalized with kwashiorkor had very low initial urinary excretions of creatinine which increased sharply during the first week of treatment. The magnitude and rates of these increases were too great to be accounted for by a change in muscle mass. The initial serum creatinine concentrations, although not initially elevated, decreased markedly parallel to the increase in urinary excretion. Endogenous creatinine clearances per square meter of body surface were consequently very reduced on admission and increased rapidly during the first week of treatment.

In patients followed up for periods varying from two to six months after initiation of treatment and who were clinically recovered, serum creatinine levels remained much lower (0.46 to 0.57 mg. per 100 ml.) than the average for a group of sixteen well nourished control subjects (0.78 mg. per 100 ml.). The average clearance for the latter children was only 68 per cent of the value found for healthy adults; it appears therefore, that creatinine clearance in children does not represent true glomerular filtration.

A practical conclusion from the results is that the figure for twenty-four-hour urinary excretion of creatinine during or soon after the acute phase of kwashiorkor, is not reliable for the estimation of muscle mass.

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Serum and Liver Vitamin A and Lipids in Children with Severe Protein Malnutrition

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SERUM cholesterol levels are very low in children suffering from the kwashiorkor type of severe protein malnutrition, but increase rapidly after a few days of therapy.[1–5] The significance of this rise became more apparent when it was found that it paralleled changes in other serum lipid constituents.[5] These increases were observed even when the therapeutic diet contained negligible amounts of fat. It has been proposed that the lipids appearing in the circulating plasma under these conditions are of tissue origin, probably from the liver,[5] although it is conceivable that some fat could have been synthesized from carbohydrates and some cholesterol from acetate.

The very reduced serum levels of vitamin A seen in children with kwashiorkor upon admission to the hospital,[6] were at first assumed to be a result of a dietary deficiency. In view of the changes in serum lipids just noted, however, the pattern of change of vitamin A serum levels during initial recovery was studied in children with kwashiorkor who received a diet practically devoid of vitamin A activity. An increase in vitamin A serum levels under these conditions could occur only at the expense of stored vitamin A since this vitamin is not synthesized *de novo* in the body. Such a finding would strongly imply that the mechanism of blood transport of lipids is impaired in acute kwashiorkor and that this impairment is rapidly corrected with treatment.

Since fat accumulates in large quantities in the liver of children with kwashiorkor, and because liver tissue is the main reservoir of vitamin A, the relative changes in the lipids and vitamin A content of the serum and liver tissue were studied during the initial phase of recovery from the disease.

MATERIAL

The subjects were boys admitted to the hospital with acute kwashiorkor, conforming to the typical clinical picture of the disease as it occurs in Central America, including edema, skin lesions, hair changes, anorexia, apathy and severe hypoproteinemia.[6]

Preliminary observations on the changes in serum levels of lipids, vitamin A and carotene were made in the three following cases:

DEC. This child, aged two years and five months, was treated from the time of admission with skim milk and calcium caseinate at a level of intake increasing from 6.6 gm. of protein and 75 cal. per kg. of body weight per day initially, to 11 gm. of protein and 94 cal. per kg. per day by the end of the one-month observation period.

VDC. This child, aged five years and six months, was given a diet of corn and beans at a level of intake of 2 gm. of protein and 70 cal. per kg. of body weight per day, for nine days following hospital admission. Forty-five per cent of the protein came from corn and 55 per cent from beans. Since the clinical condition of the child was not improving satisfactorily, the diet was changed at this time to skim milk which supplied 4.5 gm. of protein and 80 cal. per kg. per day.

BLO. This infant, aged one year and five months, was given a diet of corn and beans during the first four weeks of hospitalization at a level of intake of 2 gm. of protein and 90 cal. per kg. of body weight per day.

From the Institute of Nutrition of Central America and Panama (INCAP) Guatemala City, Guatemala. INCAP Publication I-166.

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The proportion of corn and beans was the same as for VDC. At the end of the second week, 60 gm. of a puree of a green leaf (Amaranthus hybridus) were added to the daily diet as a source of natural carotene. This supplied 5,000 I.U. of vitamin A activity. At the end of the fourth week, the corn-bean diet was replaced by skim milk, at which time the protein intake was increased gradually from 2 to 5 gm. per kg. of body weight per day and the caloric intake from 90 to 120 cal. per kg. per day. The observation period lasted two months.

In order to study changes in liver composition that accompany the changes in serum, both liver tissue samples and serum samples were studied in the following four cases:

JRA. This child, aged four years and eight months, received skim milk with calcium caseinate at a level that increased from 2 gm. of protein and 80 cal. to 5 gm. of protein and 120 cal. per kg. of body weight per day.

JRE. This child, aged three years and nine months, was treated with skim milk from the time of admission. The intake increased from 2 gm. of protein and 60 cal. to 4 gm. of protein and 90 cal. per kg. of body weight per day by the eighteenth day of study.

PAP. This three year old child was treated from the day of admission with skim milk at a level of intake which increased from 2 to 8 gm. of protein and 60 to 90 cal. per kg. of body weight per day during the observation period of three weeks.

LCA. This child, aged one year and eleven months, received skim milk from the day of admission at a level of intake of 2 gm. of protein and 60 cal. per kg. of body weight per day, increasing to 4 gm. of protein and 90 cal. per kg. per day by the end of the observation period of three weeks.

METHODS

Blood samples were obtained at approximately weekly intervals by either venipuncture or fingertip prick. The serum was separated within two hours and serum proteins were immediately estimated by the method of Lowry and Hunter.[7] Aliquots of the rest of the serum were frozen at −20°C. until analyzed. The micromethod of Bessey et al.[8] was used for the estimation of vitamin A and carotene. Cholesterol was measured by a microadaptation of the method of Abell et al.[9] using 0.050 ml. of serum. An aliquot of 0.040 ml. of serum was extracted with Bloor's solvent mixture for the determination of lipid phosphorus by the method of Maclay[10] adapted to a microscale, and total lipids by the dichromate oxidation method of Bragdon.[11] The factor of 25 was used to convert lipid phosphorus to phospholipid values.

In addition, in four cases (JRA, JRE, PAP and LCA) a liver biopsy was performed, not only before any dietary therapy was instituted, but also after satisfactory initial response to therapy, as shown by laboratory and clinical evidence. After previous verification of a normal prothrombin time and local anesthesia, the liver biopsy specimens were taken with a Franseen needle by aspiration through the pleural space. From 15 to 25 mg. of tissue were thus obtained and a 1:25 homogenate was prepared in ice cold 0.85 per cent sodium chloride solution. Not more than one hour elapsed between the taking of the sample and the homogenization, and the sample was kept in a test tube immersed in ice during that time.

Total lipids, phospholipids and vitamin A and carotene were determined in this sample by the same technics as for serum, and proteins with the reagent of Folin-Ciocalteu as described by Lowry et al.[12]

RESULTS

Table 1 gives the changes observed in the serum of the first three children studied. The clinical improvement of DEC with the milk diet was very satisfactory. His total serum proteins increased 2.2 gm. per 100 ml. in seven days and 3.6 gm. in thirteen days. The increases in phospholipids, cholesterol and vitamin A were very marked; their levels reached peak values at essentially the same time. The variations in the carotene levels in serum were insignificant.

The clinical condition of VDC did not improve satisfactorily on the corn-bean diet, and the total serum proteins did not change significantly; neither the concentration of vitamin A nor that of carotene increased during this period. Upon changing the diet to skim milk, a marked clinical improvement was seen with a parallel increase in serum proteins in ten days from 4.1 to 5.8 gm. per 100 ml. Vitamin A, phospholipids and cholesterol serum levels also increased rapidly from this time but carotene levels remained low.

On the corn-bean diet, BLO did not show appreciable recovery; some edema, irritability,

TABLE
Serum Proteins, Lipids, Vitamin A and Carotene

	DEC (Diet 1)						VDC (Diet 2)			
	Days of Treatment						Days of Treat-			
	0	7	13	20	31	40	0	6	16	22
Serum components per 100 ml.										
Proteins (gm.)...	3.2	5.4	6.8	7.0	7.0	7.4	3.8	4.1	5.8	6.3
Vitamin A (μg.).	4	37	43	30	21	8	12	9	48	47
Carotene (μg.)...	7	11	7	4	5	5	5	1	11	5
Cholesterol (mg.)..	74	219	236	...	134	94	65	...	171	144
Phospholipids (mg.)	165	320	315	...	200	135	150	...	282	238

NOTE: Diet 1—Skim milk plus calcium caseinate. Diet 2—Corn-beans for thirty days, then changed to skim milk for next thirty-five days.

TABLE
Serum and Liver Composition During

	JRA					JRE	
	Days of Treatment					Days of	
	0	7	12	15	19	3	6
Serum components per 100 ml.							
Proteins (gm.).....	3.1	4.4	5.4	5.9	6.5	3.8	4.1
Vitamin A (μg.).....	6	22	39	41	30	10	10
Carotene (μg.).....	3	5	6	6	1	5	4
Total lipids (mg.).....	520	887	940	933	840	448	502
Cholesterol (mg.).....	69	166	227	210	153	89	62
Phospholipids (mg.).....	155	252	280	255	212	120	99
Liver components per 100 gm.							
Proteins (gm.).....	13.1	...	...	...	17.3	9.3	...
Vitamin A (μg.).....	2500	...	...	...	944	1950	...
Carotene (μg.).....	47	...	...	...	51	121	...
Total lipids (gm.).....	18	...	...	...	10	...	...
Phospholipids (gm.).....	5.1	...	...	...	5.3	...	...

* All children received skim milk as the only dietary protein source.

anorexia and skin lesions were still present at the end of one month, and the serum proteins had increased only 0.5 gm. per 100 ml. Frank improvement in all the clinical signs was noted shortly after the diet of skim milk was started. The total serum proteins increased 1.1 gm. per 100 ml. in only seven days. Parallel to this improvement, serum levels of vitamin A, cholesterol and phospholipids rapidly increased; serum carotene also rose in this child.

The biochemical results of the four children in whom both changes in serum and liver composition were studied, are shown in Table II. Each of these four children was treated from the time of admission with a therapeutic diet in which the protein was supplied by milk. Except for some dehydration during the first few days, all showed satisfactory clinical recovery and their total serum proteins increased steadily with treatment.

I
During Initial Recovery from Kwashiorkor

			BLO (Diet 3)							
ment			Days of Treatment							
29	36	43	0	8	22	29	36	38	45	54
6.2	6.9	7.0	3.8	3.9	3.8	4.3	5.4	6.1	6.4	7.0
38	38	28	4	8	8	10	23	31	37	40
5	6	9	8	6	7	6	27	32	66	94
144	149	144	135	144	121	104	168	188	176	149
185	192	208	225	...	220	190	...	270	250	190

milk, green leaf carotene source added beginning on the fifteenth day. Diet 3—Corn-beans for nine days, skim

II
Initial Recovery from Kwashiorkor*

		PAP				LCA			
Treatment		Days of Treatment				Days of Treatment			
13	17	0	7	14	21	0	7	15	20
6.2	6.6	3.3	4.9	6.2	6.5	3.3	4.4	5.5	5.8
49	24	2	0	2	0	0	0	0	3
4	3	2	2	4	4	8	4	4	1
721	...	...	...	...	...	334	367	488	440
193	...	61	94	120	122	80	88	115	132
235	...	108	135	185	180	66	50	79	86
...	17.1	9.5	...	...	12.4	9.9	...	...	...
...	1090	150	...	...	0	104	...	...	...
...	116	150	...	...	97	103	...	...	...
...	7	21	...	...	8	19	...	...	...
...	4.6	3.1	...	...	3.6	3.5	...	...	...

Two of these children (JRA and JRE) had appreciable reserves of hepatic vitamin A (2,500 and 1,950 μg. per 100 gm. of fresh liver, respectively). Both vitamin A and the serum lipid fractions studied rose markedly although carotene did not. The increase in protein content of the liver was paralleled by a drop in hepatic vitamin A content in both children, while the carotene in the liver did not change appreciably.

In JRA, total liver lipids dropped from 18 per cent initially to 10 per cent on the nineteenth day with practically no variation in phospholipid content. In JRE, no initial total lipid and phospholipid analyses were possible, but the figures obtained on the seventeenth day agree with the reduced figures for JRA after initial recovery.

There were two main differences between the results in these two children and those for

PAP and LCA. In the latter, no elevation of the very low serum vitamin A levels was obtained with treatment, and furthermore, the initial vitamin A content of their liver tissue was negligible (150 and 104 μg. per 100 gm. of fresh tissue, respectively). The liver tissue analyses performed for PAP on admission and after twenty-one days of treatment, show that hepatic protein increased, carotene and fat decreased and phospholipid practically remained unchanged. No second biopsy analysis could be made on LCA, but the initial sample had a composition similar to that of PAP.

COMMENTS

With treatment, serum levels of vitamin A increased even though the diets, with one exception, did not contain vitamin A or carotene. These observations suggest that the sudden appearance of the vitamin in the circulation was due to mobilization of tissue stores brought about by protein therapy. This is confirmed by the corresponding rapid decrease in vitamin A in the liver tissue, and by the lack of serum vitamin A increases in the two children whose initial hepatic reserves of the vitamin were negligible (PAP and LCA).

By analogy, the parallel changes in total serum and liver lipids could also be explained by the improvement of an initially impaired capacity of plasma to transport them from the liver to other tissues of the body. The fact that the phospholipid concentration of liver tissue did not decrease parallel to the increase in serum, suggests that the hepatic biosynthesis of these compounds during initial recovery is sufficient to compensate for the amounts which enter the circulation.

The observed changes are closely associated with recovery, and particularly with regeneration of serum proteins. In kwashiorkor, failure to mobilize lipids and vitamin A may be a direct consequence of the severe hypoproteinemia since several fractions of the plasma proteins are important in the transport of lipid compounds. It is known, for example, that endogenous vitamin A is mobilized in the alcohol form bound to the albumin fraction of the plasma proteins.[13] Phospholipids and cholesterol are present in highest concentrations in the β-globulin fraction of proteins.[14]

Very low serum albumin levels are a universal finding in kwashiorkor and a decreased β-globulin fraction occurs frequently in Central American patients.[15] These changes may well be the primary cause of the failure in mobilization of vitamin A and lipid fractions in kwashiorkor. The rapid synthesis of the carrier-plasma proteins during recovery may be the factor determining the prompt re-establishment of normal fat transport when adequate treatment is given. This hypothesis is strengthened by the findings of Cravioto et al.[16] that serum levels of α and β-lipoproteins are initially low in kwashiorkor and increase rapidly with recovery.

When given large doses of vitamin A palmitate in oil, children with acute kwashiorkor do not show the marked increase in plasma vitamin A which occurs in well nourished subjects under these circumstances.[17] This apparent lack of absorption may well be explained by the transport disturbance indicated by the present results. In Indonesia, Yap Kie Tiong[18] found that ten children with frank keratomalacia, the most advanced eye lesion of vitamin A deficiency, presented very low levels of the vitamin as well as severe hypoalbuminemia (average 1.7 gm. per 100 ml.; range 1.3 to 2.1 gm. per 100 ml., while ten other children with only xerophthalmia also had very low serum levels of vitamin A but their serum albumin was not nearly as low (average 3 gm. per 100 ml.; range 2.5 to 3.7 gm. per 100 ml.).

The results suggest that in severe protein deficiency, the vitamin A of the diet does not reach the liver; furthermore, whatever reserves may remain in the hepatic tissue are not available to the other tissues of the body for utilization. It is thus postulated that, independently of both the dietary intake and liver stores of vitamin A, a functional deficiency of this vitamin may occur in kwashiorkor and other forms of severe protein deficiency, as a consequence of the reduced plasma proteins.

SUMMARY

The serum levels of total lipids, phospholip-

ids, cholesterol, vitamin A and carotene were measured in children with kwashiorkor on admission to the hospital, and at regular intervals during the first weeks of recovery. Marked increases in all these serum constituents were observed, except for vitamin A in some of the children. Liver biopsy specimens were also studied for the same lipid constituents, except cholesterol. The data indicate that total lipids and vitamin A decrease in liver tissue simultaneously with an increase in these serum components. The findings are suggestive of an initial impairment in lipid and vitamin A blood transport possibly associated with decreases in the plasma protein fractions to which these lipid compounds are normally bound. Phospholipids differ in that they are apparently synthesized in the liver at a rate sufficient to prevent their decrease in hepatic tissue despite increased removal in the circulation.

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Role of the Adrenal Cortical System in the Response of Children to Severe Protein Malnutrition

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KWASHIORKOR and marasmus are two contrasting clinical forms of severe malnutrition in children in technically underdeveloped areas. Among the outstanding clinical, biochemical and histopathologic characteristics of kwashiorkor are edema, severe hypoalbuminemia and fatty liver; neither edema nor fatty liver are found in marasmus and the albumin is not reduced much below normal values.[1]

The child with marasmus appears to be one whose synthesis of essential proteins, particularly those formed in the liver, has not suffered; he defends himself well through consumption of his own protein "reserves," principally muscle, and reaches a terminal state only gradually. On the other hand, in the child with kwashiorkor, some mechanism seems to have failed acutely with resulting physiopathologic alteration of a serious nature. Imbalances between anabolism and catabolism of serum albumin and of some other fundamental protein moieties, e.g., enzymes, develop rapidly in the acute phase of kwashiorkor, and are readily reversible only with appropriate therapy.

On the basis of epidemiologic observations, the appearance of one or the other of these two syndromes has been attributed to differences in the ratio of calories to protein in the diet. If the diet is poor in protein, both quantitatively and qualitatively, but has a relative excess of calories, kwashiorkor develops; if the diet is proportionally insufficient in both protein and calories, the result is marasmus.[1] This, however, does not explain the mechanism responsible for the differences in the physiopathology of kwashiorkor and marasmus. Moreover, the typical signs of kwashiorkor may be superimposed on a considerable degree of wasting and muscular loss. This condition is very frequent and is referred to as "marasmic kwashiorkor."

This study explores metabolic mechanisms which may determine which of the two clinical conditions develops in a malnourished child. When our attention was directed toward possible alterations in the endocrine system, the adrenal cortical system was selected for initial studies because of (1) its direct role in the ability of the organism to react to stress, since it is obvious that severe malnutrition is itself a basic stress, and (2) the presence of edema and electrolyte imbalance,[2-8] changes which are known to depend in part on adrenal cortex function. The endocrine system has been studied in adults with chronic malnutrition[9,10] but reports suggesting endocrine alterations in malnourished children are very limited.[11,12]

Endocrinologic observations in a group of children with either acute kwashiorkor or marasmus, and experiments with protein malnourished rats submitted to specific hormone administration, are presented.

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TABLE I
Characteristics of the Patients Studied

Group	Patients (no.)	Age (mo.)		Weight (kg.) Mean ± S.E.*	Serum Proteins (gm./100 ml.) Mean ± S.E.*	Edema
		Mean	Range			
Kwashiorkor	6	41.0	18–60	9.1 ± 1.65	3.84 ± 0.26	Present
Marasmus	5	5.6	2.5–12	3.2 ± 0.52	5.33 ± 0.32	Absent

* Standard error.

EXPERIMENTAL DATA

Observations in Malnourished Children

Subjects. Six children with kwashiorkor, and five with marasmus were chosen by clinical criteria which have been discussed elsewhere.[1] Edema was present in all the patients with kwashiorkor but not in any of those with marasmus. Some characteristics of these subjects are given in Table I.

Experimental Plan. Children with acute disease were admitted to the metabolic ward and given orally an electrolyte solution† during the first eighteen hours. A therapeutic diet of whole or half skim milk was then introduced and other foods gradually added as described previously.[13] A 10 ml. fasting venous blood sample was taken in the morning of the day following admission, 1 ml. of which was added to an anticoagulant to permit counting of eosinophils. The rest was allowed to clot and the serum separated for biochemical determinations. The child was kept in a bed equipped for quantitative urine collections and urine was collected for twelve hours in most cases and for twenty-four hours in some instances. All data on urinary excretions were calculated to twenty-four-hour periods. During collections the urine specimens were kept on ice without preservative.

Twenty-four hours after the first blood sample, an intramuscular injection of 10 I.U. of ACTH† was given and a twelve- or twenty-four-hour urinary collection was repeated. Six hours after the dose of ACTH another blood sample was taken. During the second week of hospitalization, when the patient had made major progress toward recovery, blood and urine were collected before and after stimulation with ACTH in the same manner. This procedure was repeated once more about two months later when the child was considered clinically recovered.

Studies with Rats

Material. Three separate experiments were carried out with Sprague-Dawley rats of both sexes.

Experiment 1: As a preliminary experiment, thirty rats were taken after weaning and fed Purina Laboratory Chow for one week. They were then given an experimental diet with the following composition in grams per 100 grams: ground yellow corn 61.8, corn starch 15.3, alphacel 15.3, cottonseed oil 3.5, Hegsted Mineral Mixture‡ 2.7, cod liver oil§ 1.4; vitamins were added as follows, in milligrams per 100 grams of diet: thiamine hydrochloride 3, riboflavin 3, nicotinic acid 5, calcium pantothenate 10, pyridoxine 3, biotin 0.01, folic acid 0.02, vitamin B_{12} 0.002, inositol 40, choline chloride 150, para-aminobenzoic acid 30 and menadione 1. This diet was deficient in protein both qualitatively and quantitatively, but contained all other known essential nutrients for the rat in sufficient amounts.

After twenty-one days on this diet, six animals were sacrificed following light ether anesthesia. Blood was collected and the serum separated by centrifugation for total protein and albumin determinations. The liver was observed macroscopically and a

* Lytren,® Mead Johnson & Company, Indiana.
† ACTHAR® Gel, Armour Laboratories, Illinois.
‡ Nutritional Biochemical Corp., Cleveland, Ohio.
§ 1,800 I.U. vitamin A and 200 I.U. vitamin D per gram.

section fixed in 10 per cent formaldehyde for histopathologic examination.

The remaining twenty-four rats were divided into four groups of six animals for treatment as follows: group I (control), 0.5 ml. of 0.85 per cent sodium chloride solution administered subcutaneously; group II, 3.12 mg. of cortisone acetate in aqueous suspension given subcutaneously; group III, 2 I.U. of ACTH administered subcutaneously; and group IV, 0.5 mg. of desoxycorticosterone acetate (DOCA®) in oil solution given intramuscularly. The injections were given daily to each animal for twenty-one days. At the end of this period all the rats were sacrificed and the blood and liver were sampled as before. The weights of the animals were determined throughout the experimental period.

Experiment 2: The general planning was similar to that of experiment 1 except that the rats were placed on the experimental protein deficient diet immediately after weaning. The animals were maintained on this diet throughout the experiment. After twenty-one days, six animals were assigned randomly to a "basal group" which was sacrificed to obtain pretreatment information. The remaining fifty-four rats were distributed among three groups of eighteen rats each, with each group balanced for weight and sex as nearly as possible.

The following treatments were given daily for twenty-one days: group I(control), 0.125 ml. of 0.85 per cent sodium chloride solution given subcutaneously; group II, 3.12 mg. of cortisone acetate in 0.125 ml. aqueous suspension administered subcutaneously; and group III, 1 mg. of DOCA in 0.2 ml. of oil given intramuscularly. The experimental plan called for sacrificing six animals from each group weekly. Deviations from this sampling plan were due to the deaths of two rats each in groups II and III, and the sacrifice of only five control animals at the end of the second week.

A blood sample was collected from each rat and the serum separated for measurement of total proteins, electrophoretic protein fractions and urea nitrogen. The liver was weighed and a fragment was fixed in formaldehyde for histopathologic studies. About 500 mg. of liver tissue were homogenized in ice cold distilled water to a final concentration of 1:10 in a Potter-Elvehjem glass homogenizer to determine liver moisture, protein and fat content.

The left psoas muscle was dissected out and weighed; a fragment of it was fixed in formaldehyde for histopathologic examination and another homogenized in the same way as the liver for protein determination. The viscera were then entirely removed and the water content of the carcass was determined. The animals were weighed throughout the entire experimental period, and data on food consumption were collected during a three-day period in the third week of treatment.

Experiment 3: After weaning, twenty-four rats were fed Purina Laboratory Chow for twenty-one days, and at the end of this period, six were sacrificed for the identical study described in experiment 2. The eighteen remaining animals were divided into the same three groups of six rats each. They also received the same treatments as in experiment 2 while continuing to be fed Purina Laboratory Chow for another twenty-one-day period. All the animals were sacrificed and studied as described before.

METHODS

The following methods were used: Eosinophil counting, Speirs;[14] 17-hydroxysteroids in urine, Reddy;[15] 17-ketosteroids in urine, Drekter et al;[16] serum total proteins, Lowry and Hunter;[17] serum albumin, Debro et al;[18] electrophoretic analysis, by paper electrophoresis in barbital acetate buffer pH 8.6 at an ionic strength 0.075 using 110 volts for sixteen hours with colorimetric estimation after amido-black staining and elution in 0.01 N sodium hydroxide; urea nitrogen in serum, by nesslerization after incubation with urease and precipitation of proteins with tungstic acid; total proteins in liver and muscle tissue, using the phenol reagent of Folin-Ciocalteu according to Lowry et al.;[19] moisture, drying-oven at 100°c. to constant weight; liver fat, measured in an extract of a 1:10 homogenate with Bloor's mixture by the oxidation method of Bragdon.[20]

TABLE II

Endocrine Findings in Children Suffering from Kwashiorkor and Marasmus Before and After Administration of ACTH

Test	Condition	Number of Patients			A = Initial*		B = After 2 Weeks Therapy*		C = After 8 Weeks Therapy*	
		A	B	C	Before ACTH	After ACTH	Before ACTH	After ACTH	Before ACTH	After ACTH
17-hydroxysteroids (mg./24 hr.)	Kwashiorkor	6	6	5	1.0 ± 0.27	4.60 ± 1.18	1.0 ± 0.46	3.8 ± 0.60	2.0 ± 0.71	4.1 ± 0.52
	Marasmus	5	5	3	3.3 ± 0.65	3.74 ± 0.56	1.3 ± 0.32	3.2 ± 0.49	1.4 ± 0.80	2.6 ± 0.37
17-ketosteroids (mg./24 hr.)	Kwashiorkor	6	6	5	0.5 ± 0.20	2.73 ± 3.80	1.1 ± 0.90	1.4 ± 1.08	0.6 ± 0.41	1.2 ± 0.26
	Marasmus	5	5	3	0.8 ± 0.18	1.04 ± 0.19	0.9 ± 0.42	0.6 ± 0.31	0.4 ± 0.40	0.6 ± 0.58
Eosinophil† count (per cu. mm.)	Kwashiorkor	4	6	6	82 (56–100)	...	438 (133–872)	...	631 (144–1100)	...
	Marasmus	5	5	3	9 (0–22)	...	83 (122–155)	...	119 (17–206)	...

NOTE: The twenty-four hour urinary excretions of steroids are in absolute terms and not per square meter of body surface because, due to an oversight, no accurate record of the children's height was taken. It should be emphasized, however, that since the children with marasmus were much younger and weighed much less than those with kwashiorkor, expressing the data per unit of body surface would have indicated even more dramatically the higher steroid excretions of the patients with marasmus.

* Mean ± standard error.

† Range of eosinophil counts is given in parenthesis below the mean.

RESULTS

Children. Table II gives the results of the determination of urinary excretion of steroids and the eosinophil counts of the children with kwashiorkor and marasmus. The eosinophil count was much lower in all the children with marasmus on admission, and despite their younger age they excreted significantly more 17-hydroxysteroids than the children with kwashiorkor. Both of these indices suggest an overactive glucocorticoid function in marasmus. The average 17-ketosteroid excretion was not significantly different in the children with kwashiorkor compared to those with marasmus.

After two weeks of treatment the difference in values for 17-hydroxysteroids in the two conditions was less, and after eight weeks the relationships were reversed. The significantly lower eosinophil counts in children with marasmus than in those with kwashiorkor at admission were observed also upon each subsequent examination although the values increased with treatment in both groups. On admission, the response in 17-hydroxysteroid excretion to ACTH was relatively and absolutely much more marked in the patients with kwashiorkor who also had significantly lower excretions before stimulation with ACTH. No response was observed in three of the children with marasmus and a slight response in two, suggesting an overstimulated gland in this condition even before the administration of ACTH. At the second and eighth weeks, differences in response between children with marasmus and those with kwashiorkor were no longer significant.

On admission, the 17-ketosteroids of children with kwashiorkor increased significantly after the administration of ACTH but the response in those with marasmus was relatively small and not significant. At the end of the second and eighth weeks differences in this measurement were less marked.

Rats. Experiment 1: The results of the first rat experiment are given in Table III. After three weeks on the "poor protein diet," serum proteins and albumin values were low. The administration of cortisone for three weeks resulted in a marked rise in the serum total proteins, due entirely to an increase in the albumin concentration. This finding contrasted with those for the other three groups, in which the albumin even decreased from the pretreatment value; the globulins on the other hand, had a tendency to increase, particularly in the groups treated with DOCA and ACTH.

TABLE III

Serum, Liver and Weight Findings in Protein Deficient Rats Given Adrenal Cortical Hormones or ACTH (Six Rats per Group)

Test	Pretreatment	Three Weeks Treatment			
		Sodium Chloride	Cortisone	ACTH	DOCA
Serum*† (gm./100 ml.)					
Total proteins	4.61 ± 0.16	5.10 ± 0.18	6.11 ± 0.10	5.24 ± 0.05	5.26 ± 0.10
Albumin	2.45 ± 0.17	2.29 ± 0.08	3.78 ± 0.09	2.14 ± 0.06	2.21 ± 0.07
Globulin	2.16 ± 0.09	2.80 ± 0.14	2.33 ± 0.07	3.09 ± 0.07	3.05 ± 0.10
Liver	(+)	(++)	(0)	(+)	(+++)
Fat infiltration (histopathologic evaluation with Sudan IV)	(+++)	(+)	(±)	(+)	(+++)
	(++)	(+++)	(±)	(±)	(+)
	(++)	(++)	(++)	(+)	(++)
	(++)	(+)	(+)	(+)	(+)
	(±)	(+)	(±)	(+)	(++)
Weight (gm.)†					
Initial	...	79.7 ± 3.91	77.3 ± 5.43	81.7 ± 3.77	73.4 ± 4.13
After therapy	...	107.0 ± 5.20	77.7 ± 5.64	106.7 ± 6.45	85.0 ± 7.20
Net gain	...	27.3 ± 4.38	0.4 ± 3.57	25.0 ± 4.81	11.6 ± 3.89

* Five rats only in pretreatment group.
† Mean ± standard error.

TABLE IV

Food Consumption, Body and Muscle Weights in Protein-Deficient Rats Treated with Cortisone and DOCA*

Treatment	Food Consumption (3 days total)	Body Weight (gm.)				Carcass Moisture† (gm./100 gm.)	Psoas Muscle Weight†	
		Before Therapy	1 Week	2 Weeks	3 Weeks		mg.	Per gm. of Body Weight
Sodium chloride	23.4 ± 1.86	49.1 ± 2.66	53.6 ± 2.48	55.7 ± 2.69	57.8 ± 2.71	60.2 ± 0.61	915 ± 39.0	15.9 ± 0.61
Cortisone	24.0 ± 1.76	48.0 ± 3.85	51.8 ± 4.92	50.2 ± 4.99	45.0 ± 4.54	62.9 ± 1.46	439 ± 67.7	9.6 ± 0.58
DOCA	23.4 ± 2.73	51.7 ± 1.60	56.0 ± 2.22	61.0 ± 2.31	65.3 ± 3.45	58.5 ± 0.93	825 ± 80.4	12.7 ± 0.90

* All values are mean ± standard error. For number of animals in each case see Table V.
† After three weeks of treatment.

TABLE V

Effect of Cortisone and DOCA on the Serum Proteins (gm./100 ml.) and the Serum Urea Nitrogen (mg./100 ml.) of Protein-Deficient Rats*

Treatment	Weeks of treatment	No. of Rats	Total Serum Protein†	Serum Albumin	Serum α-Globulin	Serum β-globulin	Serum γ-globulin	Serum Urea Nitrogen‡
Sodium chloride	1	6	4.30 ± 0.11	2.39 ± 0.13	1.05 ± 0.03	0.57 ± 0.04	0.35 ± 0.03	22.3 ± 1.15
	2	5	4.39 ± 0.12	2.23 ± 0.10	1.17 ± 0.04	0.67 ± 0.03	0.31 ± 0.03	24.3 ± 0.41
	3	7	4.48 ± 0.07	2.35 ± 0.13	1.14 ± 0.04	0.62 ± 0.04	0.36 ± 0.07	28.4 ± 1.27
Cortisone	1	6	5.41 ± 0.17	3.63 ± 0.18	1.02 ± 0.04	0.53 ± 0.06	0.22 ± 0.02	13.4 ± 0.35
	2	5	5.36 ± 0.20	3.45 ± 0.24	0.66 ± 0.23	0.72 ± 0.04	0.14 ± 0.01	17.0 ± 0.74
	3	5	4.95 ± 0.26	3.15 ± 0.26	0.96 ± 0.08	0.67 ± 0.06	0.16 ± 0.02	25.9 ± 4.91
DOCA	1	6	3.95 ± 0.09	2.08 ± 0.07	0.96 ± 0.05	0.69 ± 0.04	0.22 ± 0.02	23.8 ± 1.50
	2	4	3.70 ± 0.34	1.67 ± 0.52	1.01 ± 0.03	0.83 ± 0.06	0.20 ± 0.03	28.2 ± 7.25
	3	6	4.81 ± 0.20	2.10 ± 0.16	1.32 ± 0.11	0.80 ± 0.06	0.63 ± 0.13	29.0 ± 3.71

* All values are mean ± standard error.
† Before treatment six rats averaged 4.63 ± 0.15 gm./100 ml. total serum protein after five weeks on the basal diet.
‡ Pretreatment value for six rats: 19.4 ± 1.51.

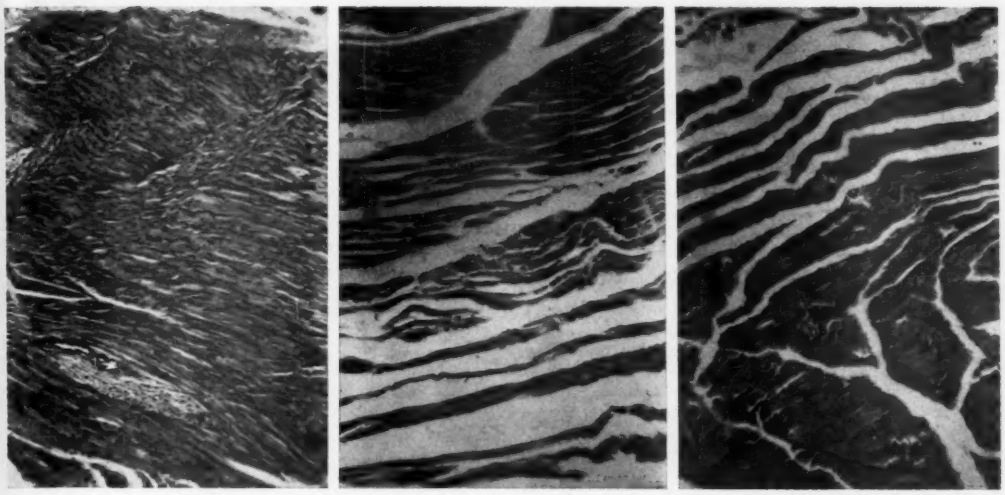

FIG. 1. Rat psoas muscle before administration of cortisone (*left*), at the second week of treatment (*middle*), and at the end of the third week of cortisone therapy (*right*). Hematoxylin and eosin stain, magnification ×100.

The results of the histologic appraisal of the liver indicate a clear-cut effect of cortisone reduction of the initial fat infiltration observed in the pretreatment group. This effect was similarly shown in the group given ACTH in contrast with the albumin regeneration effect which only cortisone produced. The animals treated with sodium chloride, and particularly those treated with DOCA, showed no decrease in hepatic fat content. Animals treated with cortisone gain practically no weight as an average, while those treated with sodium chloride and ACTH gain most.

Experiment 2: The results of the second experiment with protein deficient rats, are illustrated in Tables IV through VI. Table IV shows that the rats receiving cortisone failed to grow, while those given injections of sodium chloride solution or DOCA, gained some weight, although slowly due to the very inadequate protein intake. These results are in agreement with those of experiment 1. Carcass moisture was essentially the same in all groups.

The final weights of the psoas muscle in the three groups are entirely different and indicate a marked degree of muscle tissue wasting in the cortisone group. This is also illustrated by the much lower muscle body weight ratio shown in the last column of Table IV and by the histologic appearance of the muscle fibers (Fig. 1). At the third week of treatment the protein content of the muscle was 17 per cent for the group receiving sodium chloride, 17 per cent for the group receiving cortisone and 18 per cent for the group receiving DOCA.

The amount of food consumed was the same in the three groups. More complete data on food consumption have been collected in a subsequent experiment, during twelve-day periods with thirteen animals in each group, without finding any differences between groups.

The effects of the hormone treatments on the concentrations of serum total protein and its electrophoretic fractions and on serum urea are presented in Table V. Marked elevation in albumin by cortisone, as found in the previous experiment, was again observed. The effects on the globulins were less marked than in albumin although a significant lowering of the gamma globulin by cortisone was evident. It should be noted that at the end of the third week the serum total protein level of the group receiving DOCA approached that of the animals treated with cortisone. Nevertheless, the distribution of the protein fractions was contrasting in the two groups, the group receiving cortisone having an albumin:globulin ratio of 1:8 and the group

TABLE VI
Effect of Cortisone and DOCA on Liver Weight, Moisture, Protein and Fat (Three Weeks of Treatment)*

Treatment	Weeks of Treatment	No. of Rats	Liver Weight (gm./kg. body weight)	Moisture (gm./100 gm.)	Protein (gm./100 gm.)	Fat (gm./100 gm.)
Pretreatment	0	6	47.9 ± 1.35	69.4 ± 0.59	15.8 ± 0.50	13.6 ± 1.23
Sodium chloride	1	6	47.3 ± 1.23	69.7 ± 0.30	15.2 ± 0.26	14.2 ± 1.28
	2	5	53.3 ± 1.62	69.2 ± 0.95	13.9 ± 0.25	12.4 ± 1.07
	3	6	56.3 ± 3.10	72.4 ± 0.94	14.5 ± 0.36	8.9 ± 1.08
Cortisone	1	6	67.9 ± 3.24	69.1 ± 0.63	16.8 ± 0.54	10.2 ± 0.63
	2	5	63.9 ± 2.16	69.9 ± 0.43	17.2 ± 0.31	9.7 ± 0.42
	3	5	62.8 ± 3.05	72.1 ± 0.37	19.3 ± 0.64	7.6 ± 0.64
DOCA	1	6	55.4 ± 1.64	70.1 ± 0.23	14.8 ± 0.32	13.4 ± 0.86
	2	4	58.8 ± 6.33	71.8 ± 3.79	13.1 ± 0.54	12.5 ± 2.72
	3	6	57.1 ± 2.63	71.8 ± 0.81	14.4 ± 0.56	10.3 ± 0.88

* All values are mean ± standard error.

receiving DOCA, 0:8. The same tendency, and to almost the same degree, was observed in experiment 1. The concentration of urea nitrogen in serum was significantly depressed at the end of the first week of cortisone treatment; this very significant effect became less marked in the two subsequent weeks.

The results of the cortisone and desoxycorticosterone treatments on the liver are shown in Table VI. Both the weight of the liver per unit of body weight and the hepatic protein concentration were higher in the rats receiving cortisone as compared with those treated with sodium chloride or DOCA. The amount of fat in the liver was less in the animals treated with cortisone than in the other two groups; while the water content of hepatic tissue was essentially the same in all groups.

Experiment 3: The results of this experiment are presented in Table VII. The administration of cortisone and DOCA to the well nourished rats did not cause the effects observed in protein-deficient animals. At about forty days of age the weight of these rats was around 200 gm. compared with an average weight of about 40 to 50 gm. for the malnourished rats of experiment 2. No significant differences in any of the other biochemical or physical measurements resulted from any of the three treatments, sodium chloride, cortisone and DOCA.

TABLE
Findings in Well
(Six Rats

Group	Weight Gain (gm.)	Total Serum Protein (gm./100 ml.)	Serum Albumin (gm./100 ml.)	Serum Globulin (gm./100 ml.)
Pretreatment	†	5.41 ± 0.05	3.37 ± 0.05	2.04 ± 0.03
Sodium chloride, third week	88.0 ± 13.25	5.72 ± 0.06	3.56 ± 0.11	2.17 ± 0.13
Cortisone, third week	42.0 ± 3.48	6.24 ± 0.12	3.51 ± 0.12	2.73 ± 0.04
DOCA, third week	63.0 ± 3.70	5.69 ± 0.11	3.33 ± 0.07	2.36 ± 0.10

* All values are mean ± standard error.

COMMENTS

Nutriture does not depend on the nutrient content of the diet alone; the response of the organism to specific dietary situations is of utmost importance, and the endocrine system plays a fundamental role in the processes of distribution and assimilation of food within the body.

Our observations in malnourished children suggest that patients with marasmus have a very high glucocorticoid hormone activity while those with kwashiorkor have a low one. From the reaction of children with kwashiorkor to the administration of ACTH, it appears that their reduced glucocorticoid activity was not due to primary inability of the adrenal gland to respond.

The studies with experimental animals reported here were designed to provide more evidence on this point. The low-protein corn diet was obviously insufficient for normal growth of the rats and its amino acid deficiencies were similar to those in the diets of malnourished children in Central America. Under these conditions, cortisone was administered in order to simulate the hyperglucocorticoid activity found in the children with marasmus, and ACTH was given to obtain information about capacity of the adrenal to respond. There is evidence that the secretion of minerocorticoids continues at normal or even increased rates after hypophysectomy.[21] If this mechanism were operating in the children with kwashiorkor, it might furnish an explanation for the universal finding of edema in patients with this syndrome. The administration of DOCA to some of the rats was based on this hypothesis.

The effects of cortisone administration conform generally to the results of other investigators,[22] that is, a loss of muscle accompanied by an increase in nitrogen content of the liver. Our data demonstrate that this reciprocal relationship between protein metabolism in muscle and in liver also operates with a very high degree of efficiency in the severely protein-deficient animal. The histopathologic and the biochemical data show that *the administration of cortisone results in a complete protection from the degenerative changes due to the limiting nutritional condition applied.*

The demonstration that an efficient regeneration of plasma albumin occurred in the animals treated with cortisone may be of great significance. This effect was very rapid; a week of treatment was sufficient to produce maximum differences which persisted throughout the period of observation between the group treated with sodium chloride and the group treated with cortisone. The hepatic nitrogen increased progressively in three weeks to the level of well nourished animals. Although observations of less than three weeks were not made, the adequately nourished rats showed no evidence at the end of the third week of any sustained effect of the administration of cortisone.

These contrasting metabolic effects of cortisone on muscle and viscera protein are accompanied under ordinary nutritional conditions by an over-all increase in urea production. In this sense the much lower concentrations

VII
Nourished Rats
per Group)*

Serum Urea Nitrogen (mg./100 ml.)	Carcass Moisture (gm./100 gm.)	Liver Weight (gm./kg. body weight)	Liver Moisture (gm./100 gm.)	Liver Protein (gm./100 gm.)
20.7 ± 0.82	68.9 ± 0.36	56.3 ± 1.67	71.0 ± 0.53	19.5 ± 0.25
29.4 ± 2.49	66.1 ± 0.14	51.0 ± 3.57	74.0 ± 1.62	19.3 ± 0.91
28.1 ± 0.59	66.0 ± 0.86	51.7 ± 1.22	72.5 ± 0.24	19.8 ± 0.49
28.8 ± 1.08	65.3 ± 0.83	58.3 ± 2.63	74.7 ± 0.34	17.8 ± 0.50

† Initial average weight for all groups (eighteen rats): 138.0 ± 6.35.

of serum urea found in the protein-deficient rats receiving cortisone, particularly at the end of one week of treatment are contrary to expectations. They suggest that the process of reutilization of amino acid nitrogen in these protein-deficient organisms includes an adaptive decrease in deamination.

By analogy with the term gluconeogenesis, we may refer to the synthesis of liver protein and serum albumin from muscle amino acids as "proteoneogenesis." The evidence presented indicates that the relative extent to which gluconeogenesis or proteoneogenesis occurs in an animal under cortisone administration or endogenous glucocorticoid production will be influenced by the state of protein nutrition; the balance will be in favor of gluconeogenesis in the adequately nourished organism and in favor of proteoneogenesis in the protein-depleted animal.

The lower fatty infiltration of the liver of the rats treated with cortisone, evidenced by macroscopic, histopathologic and biochemical examination, suggests that the malnourished organism whose glucocorticoid activity allows for a reutilization of amino acids for resynthesis of proteins and not as energy sources, fills its basic energy needs by metabolizing fat. This would explain the lower fat content of the rats treated with cortisone.

Some of the tendencies shown by the rats treated with DOCA contrast with the changes produced by the administration of cortisone. Compared with the values in the control animals, plasma albumin concentrations and liver fat deviated in opposite directions in these two groups. The plasma globulins in both rat experiments were higher in the group given DOCA than in the control group, although DOCA treatment did not seem to influence other characteristics appreciably. It must be emphasized that the rats under experimentation had their adrenal glands intact so that their glucocorticoid production, although possibly reduced, may have been confusing the response. Experiments with adrenolectomized animals are now in progress.

The underlying similarity between the child with marasmus and the deficient rat receiving cortisone is a severe malnutrition accompanied by evidence of hyperglucocorticoid activity. They have the following important consequences: (1) a high degree of muscle wasting, (2) a protection against hypoalbuminemia even under conditions of severely inadequate protein intake, (3) a liver of normal appearance both macroscopically and microscopically with no marked tendency to fatty infiltration and a normal protein content. In the protein-deficient rats treated with DOCA, and to a slightly lesser extent in the control animals given sodium chloride, two characteristics found in children with kwashiorkor were observed: (1) a reduction in the concentration of plasma albumin and total proteins, and (2) fatty infiltration of the liver. The implications of these results for therapy of the malnourished child should be explored.

SUMMARY

Six children with kwashiorkor upon admission were found to excrete significantly less 17-hydroxysteroids than five with marasmus although the 17-ketosteroid excretion was not significantly different in the two groups. Eosinophil counts were low in both conditions, but more markedly decreased in marasmus. Intramuscular administration of a single 10 I.U. dose of ACTH produced a sharp rise in the excretion of 17-hydroxysteroids in children with kwashiorkor but had little effect in those with marasmus. With two to eight weeks of treatment these differences disappeared even after stimulation with ACTH.

These findings were duplicated in rats fed a low-protein diet and then given either placebo, cortisone, ACTH or DOCA. Cortisone caused a marked increase in serum albumin and liver protein and a decrease in liver fat but with the other treatments these effects were not noticed. A marked degree of muscle wasting has been demonstrated histologically and chemically in the groups given cortisone. None of the treatments caused any change in the results of the measurements in well nourished rats. Thus, the child with marasmus resembled the protein-deficient rat given cortisone while the child with kwashiorkor was

similar in several basic ways to protein-deficient rats given DOCA or sodium chloride.

By analogy with gluconeogenesis, the term "proteoneogenesis" is used to define the synthesis of liver protein and serum albumin from muscle amino acids. The present findings were interpreted as an indication of predominance of "proteoneogenesis" over gluconeogenesis in the protein-deficient animal in the presence of hyperglucocorticoid activity.

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All-Vegetable Protein Mixtures for Human Feeding

V. Clinical Trials with INCAP Mixtures 8 and 9 and with Corn and Beans

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PROTEIN DEFICIENCY is widely recognized to be the major cause of the high rates of morbidity and mortality of infants in the immediate postweaning period in most technically underdeveloped areas. Despite all measures that can be taken to accelerate the production of animals, in many of these areas, there is little prospect that enough animal protein will be available soon enough and at a sufficiently low cost to solve the entire problem.

Previous papers in this series have described the laboratory studies and biologic trials involved in the development of a product containing only vegetables which has high nutritive value and is identified as INCAP Mixture 8.[1,2] Detailed descriptions of similar work with INCAP Mixture 9, an even less expensive mixture, have since been prepared.[3,4] While designed initially for the supplementary and mixed feeding of infants and young children, both preparations are suitable for consumption by persons of all ages and are relatively inexpensive. In dry form, they contain over 25 per cent protein of a quality comparable to that of some proteins of animal origin. They also supply desirable amounts of other essential nutrients except ascorbic acid. The generic name INCAPARINA has been given to cereal mixtures of this type which are developed by the Institute of Nutrition of Central America and Panama.

The biologic trials of these two preparations and variations of them were so favorable that the metabolic and therapeutic studies described in this paper were carried out with children. As a result, these mixtures were recommended for field acceptability trials in human populations. The satisfactory outcome of both types of studies has led in turn to marketing trials of Mixture 9B.

MATERIAL AND METHODS

Children with acute kwashiorkor were admitted to the INCAP metabolic unit for experimental treatments, and these, or similar children in a late stage of convalescence, were used in the studies comparing nitrogen balances in patients receiving milk and the vegetable mixtures. All patients were between thirteen months and five years of age, except for one child who was seven years old. To simplify collection of specimens of urine and feces, only male children were selected. The administration of milk as our standard treatment of patients with kwashiorkor has already been described[5] as have been the procedures followed in metabolic balance studies.[6] Intakes

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TABLE I
Formulas for INCAP Vegetable Mixtures for Human Feeding

Ingredients	Mixture (per cent)				
	8	8A	9	9A	9B
Corn					
Lime-treated	50	50	28	...	...
Cooked	...	...	...	29	...
Uncooked	...	...	...	...	29
Sesame flour with					
33 per cent fat	35	...	...	...	...
18 per cent fat	...	35	...	...	...
Sorghum					
Lime-treated	...	...	28	...	...
Cooked	...	...	...	29	...
Uncooked	...	...	...	...	29
Cottonseed	9	9	38	38	38
Torula yeast	3	3	3	3	3
Dehydrated leaf meal	3	3	3	...	...
$CaCO_3$	...	...	...	1	1
Vitamin A (in I.U.)	...	...	...	4,500	4,500

only in using sesame flour with a lower content of fat.* Before grinding, the corn and sorghum in Mixture 9A were cooked for fifteen minutes at 5 pounds pressure† with the material being soaked to a moisture content of 25 to 30 per cent. The specifications for the Torula yeast were based on Type 200‡ which contained 0.6 mg. thiamine, 0.2 mg. riboflavin and 1.0 mg. niacin. After the completion of the trials described herein, Torula yeast Type CF2‡ was specified. This type is identical in all respects to Type 200 except for a doubled content of riboflavin.

The nutrient content of vegetable Mixture 8 has already been published;[7] that of Mixture 9B, made with the Type CF2 Torula yeast, is given in Table II. The compositions of proteins and amino acids of Mixtures 9, 9A and 9B do not differ significantly. All of the mixtures were boiled for ten to fifteen minutes with sufficient water to make a gruel known locally as "atole" and fed in this form seasoned

TABLE II
Content of Mixture 9B (per 100 gm.)

Protein 27.5 gm.	Calcium 656 mg.	Leucine 2.08 gm.
Fat 4.2 gm.	Iron 8.4 mg.	Lysine 1.09 gm.
Carbohydrate 53.8 gm.	Phosphorus 69.8 mg.	Methionine and cystine 0.82 gm.
Calories 370	Sodium 3.7 mEq.	
Thiamine 2.3 mg.	Potassium 27.9 mEq.	Phenylalanine and tyrosine 2.11 gm.
Riboflavin 1.1 mg.	Arginine 2.57 gm.	Threonine 1.18 gm.
Niacin 7.8 mg.	Histidine 0.53 gm.	Tryptophan 0.29 gm.
Vitamin A 4,500 I.U.	Isoleucine 1.11 gm.	Valine 1.44 gm.

of proteins and calories were based on estimated requirements per kg. of body weight. Supplementary ascorbic acid was given with all diets and, when frank anemia was present, ferrous sulfate was given orally after initial recovery. Intakes of dietary proteins were determined by actual analyses of the diets. Urine and feces were collected for three- or five-day periods. Nitrogen was measured by the Kjeldahl procedure.

The formulas for the various INCAP vegetable mixtures tested are given in Table I. Specifications for the ingredients in Mixture 8 were given previously[7] and Mixture 8A differs with sugar and cinnamon or other flavor. Five children were treated with a precooked form of Mixture 9B which was prepared by the same process of cooking, drying and flaking

* Both lots of sesame flour were obtained from American Sesame Products Inc., Paris, Texas, through the courtesy of Mr. John H. Kraft.

† These substances were prepared through the courtesy of the Instituto Centro Americano de Investigaciones y Tecnología Industrial (ICAITI), Guatemala, C.A.

‡ This type was supplied by the Lakes States Yeast Corporation, Rhinelander, Wisconsin.

TABLE III
Comparison of Vegetable Mixture 8 and Milk in Young Children Recovered from Kwashiorkor

No. of Children	Age	Weight (kg.)	Calories per Kilogram	Milk				Vegetable Mixture 8			
				Days	Protein Intake (gm./kg.)	Absorption (% of intake)	Retention (% of intake)	Days	Protein Intake (gm./kg.)	Absorption (% of intake)	Retention (% of intake)
48	1 yr. 9 mo.	7.5	101	5	2.5	87	0	5	2.3	73	11.3
23A	3 yr.	11.2	106	5	2.6	74	23.2	5	2.6	78	23.2
48	1 yr. 9 mo.	9.9	110	5	3.8	90	14.8	5	3.8	71	12.0
56	3 yr. 6 mo.	9.5	101	5	2.8	74	11.0	5	3.0	71	13.5
57	4 yr. 3 mo.	11.6	108	5	2.9	80	33.6	5	2.8	68	22.5
73	1 yr. 1 mo.	7.4	100	5	3.0	83	17.6	10	3.5	78	31.3
75	1 yr. 3 mo.	7.5	95	10	2.6	83	38.2	5	2.9	70	23.8
88	3 yr.	10.7	108	5	2.0	78	25.9	10	3.3	67	15.2
			(104)*		(2.8)	(81)	(20.5)		(3.0)	(72)	(19.1)

* Numbers in parentheses are averages.

employed in the manufacture of commercial "instant" baby cereals.*

Efforts were also made to treat three children with kwashiorkor with a diet containing lime-treated corn in the form of atole and tortillas and cooked black beans (Phaseolus vulgaris) in which 43 per cent of the protein came from the former and 57 per cent from the latter. Biologic values of this corn and bean mixture, of milk and of Mixture 9B were each measured in a single child by the method of Mitchell.[8]† Also included in this report are preliminary data obtained from a series of trials in which either Mixture 8 or 9 was fed, both with and without fat, and nitrogen absorption and retention compared.

RESULTS

Tolerance and Acceptability

Before prolonged trials of feeding Mixture 8 *per se*, it was added to the mixed diets of eight children who had recovered from the acute phase of kwashiorkor.[9] No gastrointestinal disturbances were observed. It was then given as the sole source of protein to two children, who were completely recovered from kwashiorkor, and later to five children, who had passed the acute phase but were not yet ready for discharge from the hospital.[7,9] These children received Mixture 8 for periods of 12, 33, 41, 59 and 50 days, respectively. In every instance, the clinical response was excellent, and the mixture seemed more effective than milk in reducing the tendency of diarrhea by these subjects. Therefore, it was deemed safe to proceed with therapeutic trials of Mixture 8 in cases of patients with acute kwashiorkor.[7,9]

The tolerance of patients to Mixture 9 was established in the nitrogen balance studies in three children and by administration of the mixture to two children who had partially recovered from kwashiorkor. The employment of this formula in the treatment of children with acute kwashiorkor followed promptly. Subsequent to the studies of nitrogen balance and treatments reported in this paper, the long term tolerance to and acceptability of INCAP Mixtures 9A and 9B were confirmed through field studies in four Guatemalan communities.‡ The children in these com-

* Furnished by the "Junket" Brand Foods Division of Christian Hansen's Laboratory, Little Falls, New York, through the courtesy of Theodore F. Irmiter, M.D.

‡ Supervised by Dr. Romeo de León, Nutrition Section, Public Health Department, Guatemala.

† Biologic value $= \frac{\text{N intake} - (\text{fecal N} - \text{metabolic N}) - (\text{urinary N} - \text{endogenous N})}{\text{N intake} - (\text{fecal N} - \text{metabolic N})}$.

TABLE IV
Comparison of Vegetable Mixture 9B and Milk in Young Children Recovered from Kwashiorkor

Gram Protein per Kilogram	No. of Children	No. of Balance Periods	Milk			Vegetable Mixtures 9A or 9B		
			Protein Intake (gm./kg).	Absorption (% of intake)	Retention (% of intake)	Protein Intake (gm./kg.)	Absorption (% of intake)	Retention (% of intake)
>4.0	1	2	4.0	84.4	22.0	4	66.1	24.1
3.0–3.9	4	11	3.0	84.9	17.1	3.0	70.2	16.8
2.0–2.9	9	48	2.3	82.6	16.3	2.3	68.9	17.8
1.0–1.9	4	13	1.2	78.1	24.9	1.2	66.2	15.5
<1	2	3	0.5	67.2	8.1	0.5	59.1	4.5

munities consumed one of these formulas daily for periods of 15 to 19 weeks. During any given week, an average of 78 per cent of all children scheduled to receive the mixture, consumed two or more glasses daily. No instances of intolerance to the mixture were encountered. Ninety-seven per cent of all offerings were accepted and only 3 per cent rejected. Subsequently, similar results were obtained in a study of fifty-three children given Mixture 9B for four weeks in El Salvador.* From March 22 to July 22, 1960, a total of 485,000 packages of 75 gm. of INCAPARINA, made according to formula 9B, have been consumed in a marketing trial in Guatemala with a very good acceptability and no reports of intolerance.

Nitrogen Balance Studies

All results of the nitrogen balance studies carried out during intake of INCAP vegetable Mixture 8 are summarized in Table III, including results obtained from five patients which have been published previously.[7] Although there are variations in the relative retention of nitrogen during intake of either milk or Mixture 8, they seem to be random; thus there is no significant difference between the average retention with milk and that with Mixture 8. However, a consistently higher percentage of the protein intake is absorbed during the periods of milk intake, so that the percentage of absorbed nitrogen retained by the child is higher with vegetable Mixture 8 than with milk. Three patients also received Mixtures 8 and 8A at about the same levels of protein intake. In two of these, PC-83 and PC-88, results were comparable, i.e., 16.8 and 15.2 per cent retention for Mixture 8 and 13.9 and 14.1 per cent for Mixture 8A. For unknown reasons, the apparent retention in the third patient (PC-85) was 21.3 per cent with Mixture 8 and 4.7 per cent with Mixture 8A.

Many more balance studies were carried out with Mixtures 9, 9A and 9B than with Mixtures 8 and 8A because of increasing indications of the practical value of the former in Central America. The results of nitrogen balance tests are summarized in Table IV by level of protein intake, making no distinction among the three variations of Mixture 9. At adequate levels of protein intake, i.e., 2 gm. per kg. and above, no significant differences between the amounts of nitrogen retained were observed during intake of milk and that of INCAPARINA. At lower levels of intake, retentions with milk were significantly better, although the retentions with Mixture 9 were still positive.

Therapeutic Trials

Mixture 8. The case histories of the first three children with kwashiorkor, PC-65 (E.P.L.), PC-66 (J.A.Q.) and PC-67 (C.A.P.), who were treated with Mixture 8 from the time of admission to the hospital, have already been published.[7] In all three cases, the mixture was well accepted, and recovery was prompt

* Supervised by Dr. Amanda E. Castillo de López, Nutrition Service, Public Health Department, El Salvador.

and complete. The results were indistinguishable from those obtained from comparable patients who were treated with milk, except for a slightly slower regeneration of serum protein.[10]

The case histories of two additional children with acute kwashiorkor are cited below. The first of these, PC-73, was treated from the beginning with Mixture 8, while PC-88 was treated alternately with Mixtures 8A and 8; both recovered uneventfully. A sixth patient, PC-85, who was treated from the beginning with Mixture 8A, recovered normally from kwashiorkor but died subsequently of aspiration pneumonia during recovery from measles. No intolerance to Mixture 8 was noted at any time. However, in two instances, children PC-83 and PC-84 who were initially treated with Mixture 8A for ten days and subsequently treated with Mixture 8 for one month, the treatment with Mixture 8A was discontinued because of excessive vomiting.

PC-73, a boy one year of age, was admitted with severe kwashiorkor; he weighed 7.9 kg. and measured 68 cm. The level of total proteins in his serum at the time of admittance was 3.6 gm. per 100 ml. He was given Mixture 8 at a level of about 2 gm. protein per kg. body weight per day. This was rapidly increased in order to provide an intake of 3 gm. protein and 100 calories per kg. per day by the third day of treatment. Two weeks after admission, the edema had disappeared and he weighed 6.4 kg. After two months on the vegetable mixture, he was considered clinically recovered. His weight at this time was 7.3 kg., and the level of total proteins in his serum was 6.4 gm. per 100 ml.

PC-88, a boy two years and eight months of age, was admitted with moderate kwashiorkor; he weighed 8.4 kg. and measured 76 cm. The level of total proteins in his serum at the time of admittance was 3.4 gm. per 100 ml. Treatment was initiated with Mixture 8A at 2 gm. protein and 60 calories per kg. body weight per day. This amount was increased gradually to a level of 4 gm. protein and 110 calories per kg. per day by the ninth day. Edema began to disappear by the fourth day and the skin lesions were definitely improved by the sixth day. After two weeks, the edema had disappeared completely. The child's minimum weight at this time was 7.4 kg. and there was good improvement of all the other signs and symptoms. In view of the fact that he vomited frequently, he was changed to Mixture 8 which he accepted well without further vomiting. He was kept on this diet for two weeks and then again given Mixture 8A during which time there was no further vomiting. He was kept on Mixture 8A for eight additional weeks by which time complete clinical recovery and a weight of 10 kg. had been attained. The level of total proteins in his serum ten weeks after admission was 6.6 gm. per 100 ml. During treatment he developed measles and recovered uneventfully.

PC-85, a boy one year and eight months of age, was admitted with moderate kwashiorkor; he weighed 7.4 kg. and measured 68 cm. The level of total proteins in his serum was 3.5 gm. per 100 ml. Treatment was initiated with vegetable Mixture 8A at an intake of 2 gm. protein and 60 calories per kg. body weight per day, which was gradually increased to 3.5 gm. protein and 100 calories per kg. by the end of the second week. After seven weeks, edema and deficiency signs had disappeared and satisfactory clinical recovery had been attained. His weight was then 7.1 kg. and the level of total proteins in his serum was 5.5 gm. per 100 ml.

Mixture 9. In addition to the satisfactory results of the metabolic studies, beneficial experience was gained from our treatment of PC-88 and PC-94 before the mixture was used to treat patients with acute kwashiorkor.

PC-88, a boy two years and eleven months of age, was clinically and biochemically completely recovered from kwashiorkor. He weighed 10 kg. Balance studies were begun using other sources of protein. As a result of an accidental fall three months later, he suffered a fracture of the right femur. At this time his weight was 11.3 kg. and his general condition was satisfactory. He was then placed on a diet in which the only source of protein was Mixture 9, with an intake of 3 gm. and later of 3.5 gm. protein and 100 calories per kg. body weight per day. While on this diet he remained in a cast for a period of ten weeks. Satisfactory healing of the fracture occurred. His general condition remained satisfactory, and he gained 1.2 kg. of weight.

PC-94, a boy one year and eleven months of age, was admitted with severe kwashiorkor; he weighed 7.4 kg. and measured 77 cm. The level of total proteins in his serum was 3.4 gm. per 100 ml. He was treated with a diet based on milk and given other therapy as necessary. Three weeks after admission when he was placed on a diet of Mixture 9, he had no edema and the level of proteins in his serum was 5.7 gm. per 100 ml. His skin lesions and other manifestations of deficiency had improved considerably. He was thin, however, weighing only 6.8 kg. and in addition had a decubitus ulcer measuring 8 by 9 cm. which exposed the periosteum of the sacrum. His initial intake was 3 gm. protein and 100 calories per kg. body weight per day which was progressively increased in view of his good appetite and acceptance to 5 gm. protein and 150 calories per kg. A multivitamin-mineral prepara-

tion was administered as a dietary supplement. The level of total proteins in his serum continued to increase after Mixture 9 was introduced, and reached 6.6 gm. per 100 ml. two months after admission. At the end of three months he was a happy active child with no clinical signs of nutritional deficiency. He weighed 10 kg., a gain of 3.2 kg. over his minimum weight in nine and a half weeks with Mixture 9B. His decubitus ulcer healed spontaneously without need of grafting or other surgical procedures.

Two children with kwashiorkor, PC-95 and PC-101, were subsequently treated with precooked Mixture 9B in the INCAP metabolic unit. Results were satisfactory although recovery was complicated by an upper respiratory infection in the former and by vomiting in the latter. In order to obtain information more rapidly than would have been possible if the therapeutic trials had been limited to the INCAP metabolic unit, three children with kwashiorkor, EM9-1, EM9-2 and EM9-3, were treated with satisfactory results in the Guatemala General Hospital for one month.* Since the conditions varied, the recovery of these three patients could not be compared directly with those treated in our metabolic unit with either milk or mixtures of vegetables. The case histories of all five children are the following.

PC-95, a boy two years and nine months of age, was admitted with moderate kwashiorkor; he weighed 9.1 kg. and measured 80 cm. The level of total proteins in his serum was 3.6 gm. per 100 ml. During the first week of hospitalization, the child suffered from an upper respiratory infection. He was given precooked Mixture 9B for three weeks, at an intake that varied from 2 to 3.5 gm. protein and 80 to 120 calories per kg. body weight per day. At the end of two weeks, the edema had disappeared and his weight was 8.0 kg. Three weeks after admission, he was changed to regular Mixture 9B at a protein intake that varied from 3.5 to 5 gm. and 120 to 150 calories per kg. per day. One month after admission, the level of total proteins in his serum had reached 5.7 gm. per 100 ml. and his weight 8.6 kg. At this time, signs and symptoms of kwashiorkor had almost completely disappeared. Two months after admission, his weight had reached 9.9 kg. and the level of total proteins in his serum was 7.0 gm. per 100 ml.

PC-101, a boy five years and two months of age, was admitted with moderate kwashiorkor; he weighed 12.6 kg. and measured 92 cm. The level of total proteins in his serum was 3.0 gm. per 100 ml. He was given Mixture 9 at an intake of 2 gm. protein and 80 calories per kg. body weight per day, which was promptly increased to 3 gm. protein and 90 calories. His protein intake was reduced a week later to 2.5 gm. per kg. because of vomiting, and kept at this level until the fifth week of hospitalization when it was gradually increased to 4.0 gm. per kg. On the tenth day, he was changed to precooked Mixture 9B. His edema began to disappear by the fourth day and was gone by the seventeenth day at which time his weight was 10.8 kg. At the end of the sixth week, the level of total proteins in his serum was 6.3 gm. per 100 ml. and at two months his weight was 13.8 kg.

EM9-1, a boy five years old, was admitted with moderate kwashiorkor; he weighed 13.0 kg. and measured 92 cm. The level of total proteins in his serum was 3.8 gm. per 100 ml. He was started on precooked Mixture 9B at an intake that increased from 1.7 gm. protein and 95 calories to 2.96 gm. protein and 129 calories per kg. body weight per day. On the third day of hospitalization this child had serious diarrhea with severe dehydration which required parenteral rehydration and other measures. He was continued on Mixture 9, however, and recovered satisfactorily. His weight after ten days of treatment and loss of edema was 10.4 kg. At the end of one month, the child's level of total proteins in his serum was 6.2 gm. per 100 ml., and his weight at the end of five weeks was 12.1 kg.

EM9-2, a boy two years and two months old, was admitted with severe kwashiorkor; he weighed 7.8 kg. and measured 72 cm. The level of total proteins in his serum was 2.9 gm. per 100 ml. He was started on precooked Mixture 9B at an intake of 1.3 gm. protein and 75 calories per kg. body weight. This was increased gradually to 5 gm. protein and 150 calories per kg. body weight. After eight days of treatment, although his edema had practically disappeared, the hyperpigmentation had increased and the skin lesions had become more extensive. On the eleventh day of hospitalization, cheilosis and cheilitis were more evident than at the time of admission. At the end of two weeks, his skin was clear and the oral lesions had disappeared. His dry weight after sixteen days of treatment was 6.1 kg., remaining practically stationary for the remainder of the period of observation. On the sixteenth day of hospitalization, an abscess developed on his right arm. During virtually the entire period of treatment, he had a chronic upper respiratory infection and elevations in temperature varying up to 40°c. At the end of one month, he weighed 6.4 kg. and the level of total proteins in his serum was 4.7 gm. per 100 ml. The child had progressed satisfactorily but because of the numerous

* The cooperation of Dr. Ernesto Cofiño and Dr. Carlos Manuel Monzón Malice of the Pediatrics Service, General Hospital, Guatemala, is gratefully acknowledged.

complications he did not achieve the same state of health as the preceding patient.

EM9-3, a boy three years old, was admitted with edema and moderate kwashiorkor, weighed 9 kg. and measured 78 cm. The level of total proteins in his serum was 3.5 gm. per 100 ml. Precooked Mixture 9B was administered at an intake of 1.8 gm. protein and 100 calories per kg. body weight per day which was gradually increased to 4 gm. protein and 120 calories per kg. Although his edema began to disappear after two days of treatment, it had not completely disappeared in eleven days; he was still apathetic and anorexic and had to be fed by gavage. He also had a chronic upper respiratory infection, and after three weeks of hospitalization developed styes, first in the left eye and later in the right eye. His weight after twenty-eight days of treatment was 7.8 kg., 0.5 kg. over his minimum weight. The level of total proteins in his serum was 4.9 gm. His recovery at the end of thirty days was still incomplete but not inconsistent with that which might have occurred with milk in a case with similar complications.

Corn and Beans. In order to determine whether or not mixtures, known to be definitely inferior to milk in the quality of protein, would give a satisfactory therapeutic response in patients with kwashiorkor if a sufficient quantity of protein could be administered, attempts were made to treat three children, PC-89, PC-90 and PC-91, with a mixture in which approximately half of the protein was furnished by corn and the rest by beans. As the following case histories indicate, this failed completely and such experiments were discontinued.

PC-89, a boy one year and five months of age, was admitted with moderate kwashiorkor; he weighed 7.7 kg. and measured 70 cm. The level of total proteins in his serum was 3.8 gm. per 100 ml. After one day on electrolytes, he was placed on a diet of corn and beans (corn protein:bean protein ratio [43:57]) at an intake of 2 gm. protein and 90 calories per kg. body weight per day. A month after admission his diet was changed to skim milk at the same level of protein and calorie intake. At this time the level of total proteins in his serum was 4.3 gm. per 100 ml. and his weight was 7.1 kg. Some edema was still present. His skin lesions had not cleared completely and he still showed irritability and some apathy. During this time, cheilosis and cheilitis appeared. It was found impossible to cure this patient with a diet of corn and beans.

PC-90, a boy three years and five months of age was admitted with moderate kwashiorkor; he weighed 9.4 kg. and measured 80 cm. The level of total proteins in his serum was 5.3 gm. per 100 ml. After one day on electrolytes, he was given an ad libitum diet of corn and beans (43:57) at a level up to 2 gm. protein and 90 calories per kg. body weight per day. Because of failure to improve after twelve days, he was given a milk diet. At this time the level of total proteins in his serum was 5.0 gm. per 100 ml. and his weight was 9.1 kg. No improvement in mental symptoms had occurred; skin and mucous lesions and edema were also present.

PC-91, a boy five years and six months old, was admitted with moderate kwashiorkor; he weighed 11.4 kg. and measured 86 cm. The level of total proteins in his serum was 3.8 gm. per 100 ml. After one day of electrolytes, he was placed on a diet of corn and beans (43:57) at an intake of 2 gm. of protein and 70 calories per kg. body weight per day. Because of failure to improve, he was changed to milk one week later.

Biologic Value Determinations

Studies have also been initiated to determine the biologic value of Mixture 9B, milk and the combination of corn and beans. The experimental design required a nitrogen-free diet to be fed for four days, and the test mixture at an intake of 0.5 gm. protein per kg. per day for four additional days following a four day adaptation period. Caloric intake was held constant at 90 calories per kg. per day. During the "nitrogen-free period," actual intake of nitrogen by the three children studied to date varied between 16 and 21 mg. per day, and the nitrogen absorbed varied from 2 to minus 6 per cent. Under these conditions, the calculated biologic value for milk in child PC-101 was 92 per cent, for Mixture 9B, in PC-95 it was 88 per cent and for the combination of corn and beans in PC-91, it was 51 per cent.

Effect of Fat on Nitrogen Retention

Systematic investigations of the effect of fat on nitrogen balance are still in progress in the INCAP metabolic unit, but preliminary results seem to indicate that nitrogen retention is not influenced by the presence or absence of fat at the levels of protein and caloric intake maintained in these studies. In eleven pairs of trials of vegetable mixtures, both with and without fat, average nitrogen retentions were 12.9 per cent and 13.8 per cent of intake, respectively; the difference was not statistically

significant. Protein intakes in these comparisons varied from 1.9 to 4.3 and averaged 2.7 gm. per kg. body weight.

COMMENTS

The foregoing data demonstrate that, when the protein intake is adequate for the needs of the child, the body is able to retain as much nitrogen from the vegetable mixtures tested as from an equivalent amount of protein in milk. The results obtained in the treatment of patients with kwashiorkor also indicate that the protein of the vegetable mixtures is of relatively good quality. Nevertheless, the absorption of nitrogen as per cent of intake is always significantly less with the vegetable mixtures than with milk. Data are also presented which suggest that during inadequate intakes of protein proportionately more nitrogen is retained from protein in milk.

Since biochemical analyses have shown the vegetable mixtures tested to be deficient in several amino acids in comparison to milk or the amino acid pattern of the FAO reference protein,[10] it is not immediately clear why they should give such good results both in previous trials with rats and chicks and in the present studies with children. It would appear that a more favorable pattern of amino acids is absorbed by the child or experimental animal than is measured by microbiologic analysis following acid hydrolysis. It is perhaps significant in this regard that more of the nitrogen consumed in vegetable mixtures remains unabsorbed than when milk is the source of protein.

Direct evidence that Mixture 9B is comparable to milk in the pattern of amino acids absorbed from the gastrointestinal tract is provided by the studies of Arroyave et al.[11] They found that the pattern of amino acids in serum after a standard test meal was similar, whether milk or Mixture 9B furnished the protein in the diet, and that it was quantitatively and qualitatively different when a combination of corn and beans furnished the dietary protein.

Since the proportions of ingredients in INCAP Mixtures 8 and 9 were determined after extensive biologic trials with chicks, rats and pigs, it is not surprising that they represent efficient combinations of the ingredients. It should be noted, however, that vegetable proteins can be combined in such a way that their protein value as fed is superior to that predicted from their contents of amino acids alone.

It is theoretically possible that the protein-depleted child is less selective in his needs for nitrogen, and perhaps can use efficiently, patterns of amino acids which would not give as good results in well nourished persons. We do not know of any evidence to support such a hypothesis, and the present studies indicate that regardless of the total protein intake even the relatively good pattern of amino acids of corn and beans, compared to that of corn alone, was not sufficient for the treatment of children with kwashiorkor. Brock and Hansen report a similar outcome of their efforts to treat patients with kwashiorkor with a mixture of corn and cowpeas; satisfactory "initiation of cure" process was obtained only when corn germ was added to the combination.[12] Furthermore, experimental studies with animals have established firmly that differences in the quality of proteins are best detected through the repletion thereof in an animal which has been depleted of proteins.[13]

As previous studies of children fed corn diets supplemented with amino acids demonstrate, at low levels of protein intake deficiencies and imbalances of amino acids have a more pronounced adverse effect on nitrogen retention.[14] Therefore, it is to be expected that differences in the quality of protein between corn and Mixture 9 and its variations, which would not be apparent at relatively high levels of intake, may be detectable at low levels. This is confirmed in the data presented.

For the practical purpose of preventing protein malnutrition, all that is required of a vegetable mixture is a sufficient protein quality and concentration to be effective in the form in which it is to be consumed.

It should be stressed that all of the clinical trials have been with children over one year of age and that the mixtures have not yet been tested as milk substitutes for the artificial

feeding of infants. Although they may prove suitable for this purpose, they have been developed for the supplementary and mixed feeding of infants and young children and as a low cost source of good quality protein for persons of all ages in areas where protein of animal origin is expensive or in short supply.

The data presented indicate that both Mixture 8 and Mixture 9 and its variations meet these criteria and can be highly recommended for the intended purpose. For reasons previously reported, Mixture 9B is the most economical of the formulas for the Central American area.[15] It is already in widespread use in Guatemala under the generic name INCAPARINA, and should prove useful wherever cottonseed flour suitable for human consumption can be made available at low cost.

SUMMARY

Low cost vegetable mixtures containing over 25 per cent protein, intended for use in the prevention of protein malnutrition in technically underdeveloped areas, were fed experimentally to young children. These mixtures had been previously demonstrated to have a relatively high protein value by biologic trials with experimental animals. Mixture 8 contained 50 per cent lime-treated corn, 35 per cent sesame flour, 9 per cent cottonseed flour, 3 per cent Torula yeast and 3 per cent dehydrated leaf meal. Mixture 9B, the more practical for Central America, included 29 per cent each of uncooked ground corn and sorghum, 38 per cent cottonseed flour, 3 per cent Torula yeast, 1 per cent $CaCO_3$ and 4,500 I.U. vitamin A acetate.

In eight children, each fed at an average protein intake of 2.8 gm. per kg. per day for five day balance periods, 19.1 per cent of the nitrogen intake was retained with Mixture 8 and 20.5 per cent with milk. In nine children, forty-eight balance periods on each diet at a protein intake between 2.0 and 3.0 gm. per kg. per day gave an average retention of 16.3 per cent for milk and 17.8 per cent for Mixture 9B. In thirteen balance periods at higher levels of intake, nitrogen retentions were essentially the same for milk and Mixture 9B; but in sixteen periods at levels of 1 gm. per kg. per day and below, retentions with milk were higher. No effect of fat on nitrogen retention was detected when it was added to either of the mixtures.

Six children with kwashiorkor were treated with Mixture 8 or a lower fat variation, Mixture 8A, and five children were treated with Mixture 9B. Results were similar to those previously obtained with milk. In field trials, involving 115 children in Guatemala for fifteen to nineteen weeks and fifty-three children in El Salvador for four weeks, approximately 97 per cent of the offerings were accepted and only 3 per cent rejected. On the basis of the extensive and favorable biologic and clinical findings, both mixtures are recommended for the supplementary and mixed feeding of infants and young children and as low cost protein-rich foods of good quality for persons of all ages in any area where the basic ingredients are available and where protein of animal origin is expensive or in short supply.

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Diet and Serum Cholesterol Levels Among the "Black Caribs" of Guatemala

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SERUM cholesterol levels have been found to be markedly reduced among lower socioeconomic urban groups in Guatemala,[1-3] and the incidence and severity of atherosclerotic heart disease in these people is also very low when compared with that in business and professional persons.[4,5] The people in the rural lower socioeconomic groups in Guatemala are predominantly Mayan Indian in racial origin while those in the upper income groups are primarily European caucasoid and mestizo. This racial difference is not considered responsible for the findings, however, since similar differences in atherosclerosis are found between racially homogeneous persons in the upper and lower income groups in Costa Rica.[2,5] Environmental conditions must, therefore, be responsible; among the environmental variables which have received special attention are dietary differences.

In general, the population in the lower socioeconomic groups of this area consumes little fat, and this almost entirely of vegetable origin.[2,3] They also have low intakes of animal protein, vitamin A and riboflavin.[6] In contrast, the population in the upper socioeconomic groups in Guatemala consumes a diet relatively higher in fat and in other essential nutrients, and similar in quantity and quality to the average diet in North America.[7]

It is frequently suggested that in comparisons between different socioeconomic groups, factors other than diet may be of major importance and that the correlation with diet is secondary rather than primary. The present study is concerned with another racial group found in significant numbers in Central America, and known as the "Black Caribs."[8,9] This group, spread along the Atlantic Coast of Central America, lives at a social and economic level similar to many Mayan Indians, except for a diet much higher in proteins and fat. They also make greater use of coconut and seafood than other groups in Guatemala. The Black Caribs, therefore, provide an opportunity to determine in people living under poor socioeconomic conditions the effect on serum cholesterol levels of those dietary habits which customarily characterize upper income groups.

MATERIAL AND LOCALITY

A total of eighty-eight males and ninety-four females ranging in age from six to eighty-nine years were studied. For most decades of age it was possible to study ten persons of each sex as planned. These persons speak both Carib and Spanish and many also know English. They lived in Livingston, Guatemala, a town at the mouth of the Rio Dulce river, isolated from railroad and highway connections with the interior of Guatemala and an hour's ride by boat from the Atlantic port of Puerto Barrios. The mean annual temperature is 25.8°c. All were persons with black skin and other physical features of West Indian and African Negroes,

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TABLE I

General Characteristics and Serum Cholesterol Levels of Black Caribs in Livingston, Guatemala

Age (yr.)	No.	Weight (lb.)		Height (in.)		Skin-fold (mm.)		Blood Pressure (mm. Hg)				Serum Cholesterol (mg./100 ml.)	
								Systolic		Diastolic			
		Mean	S.D.*	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Males													
6 to 12	10	52	11	48	3	5	3	...	..	..	..	140	20
13 to 19	10	119	26	64	5	7	2	123	9	74	5	162	26
20 to 29	13	145	13	68	2	6	2	117	15	73	11	173	60
30 to 39	8	143	21	68	4	5	2	130	15	79	8	180	35
40 to 49	10	146	16	66	3	5	1	129	17	74	12	181	28
50 to 59	12	136	18	66	3	4	1	138	33	80	15	184	42
60 to 69	10	143	33	66	3	5	2	140	25	82	14	175	40
70 to 79	11	125	21	65	2	4	1	143	20	83	8	170	28
80 to 89	4	134	..	67	..	5	..	142	..	85	..	197	..
Females													
6 to 12	10	72	28	54	6	8	7	...	..	..	..	159	40
13 to 19	11	100	12	60	3	11	3	120	12	74	10	174	29
20 to 29	10	120	18	63	3	12	8	120	9	75	10	174	26
30 to 39	11	146	32	63	2	13	10	124	16	74	9	168	36
40 to 49	13	134	23	62	2	12	6	138	21	86	12	190	23
50 to 59	13	124	25	60	2	7	7	160	27	89	18	222	87
60 to 69	12	128	28	61	3	10	8	150	28	89	18	198	51
70 to 79	12	119	21	62	2	11	7	158	29	91	16	244	59
80 to 89	2	109	..	62	...	8	..	185	..	95	..	234	..

*S.D. = standard deviation.

and all belonged to a social and racial group identified as "Black Caribs." Members of the community known to represent Indian, white and oriental admixtures were excluded from the present study.

METHODS

The clinical examinations were carried out during May 1957, and included measurements of body weight, height, skinfold and blood pressure. After fasting, blood samples were extracted from the finger tips of these subjects for cholesterol analysis and were also used for determination of ABO blood type. The clinical examination followed a standard technic for nutrition surveys described by Muñoz and Pérez-Avendaño.[10] The dietary information was obtained through daily interview for seven consecutive days of families taken at random and calculated using the INCAP food composition tables.[11] The intake of fatty acid was calculated from the tables of Hardinge and Crooks.[12] The first dietary survey in December 1957 was made in the middle of the dry season; the second survey in June 1958 in the middle of the rainy season.

Total serum cholesterol levels were determined by the method of Abell et al.[13] using 0.05 ml. samples. Skinfold thickness measurements were made in triplicate over the midtriceps, using the Keys' caliper and following the recommendations of the Committee on Nutritional Anthropometry of the Food and Nutrition Board of the National Research Council (U.S.).[14] The standard slide agglutination technic was used for ABO blood typing, and genotype frequency distribution was calculated using formulas cited by Mourant.[15]

TABLE II
Blood Group Frequencies Among Black Caribs in Livingston, Guatemala

Phenotype		Genotype	
Group	Frequency (%)	Gene	Frequency (%)
O	52.3	O	72.32
A	19.9	A	13.69
B	22.2	B	15.03
AB	5.7	...	...

RESULTS

The mean and standard deviation of values for weight, height, thickness of skinfold over the triceps, blood pressure and serum cholesterol are given in Table I. Average heights and weights for each decade beyond thirty years of age remained relatively constant. Skinfold thickness was remarkably constant among adults of both sexes, although the women showed both higher values and greater variation. Women over thirty years of age also had consistently higher mean blood pressure readings. Almost half of those over forty years of age had blood pressure readings above 140/90 mm. Hg, and twelve of ninety-nine had values on or above the lower limits of hypertension by the criteria of Master et al.[16]

Serum cholesterol levels were higher in females at all ages and showed a tendency to increase in women over fifty years of age. There were three cases of hypercholesterolemia in the 182 subjects studied. One, a twenty-three year old man, had a value of 339 mg. per 100 ml. The other two were women fifty-six and seventy years of age, with 452 and 369 mg. per 100 ml. of serum cholesterol, respectively. Analysis of variance indicated that the over-all differences between men and women were significant for all measurements, as were the age trends among adults for weight and blood pressure, but not for skinfold thickness or serum cholesterol levels.

The ABO phenotype distribution and the calculated genotype frequencies are shown in Table II. The frequency of blood type O was 52.3 per cent, those of types A and B were 19.9 and 22.2 per cent, respectively, and type AB was found with a frequency of 5.7 per cent.

Table III gives the average daily dietary intake of the Black Carib population. Adequate calories, total protein and animal protein were consumed. Of the 67 gm. of total fat, 33 gm. were of animal, and 34 gm. of vegetable origin. Coconut fat contributed 13 gm. daily. Thirty-one per cent of calories were derived from fat, 6 per cent of which came from coconut fat. The saturated, monounsaturated and polyunsaturated fatty acids in the diet amounted to 28.7, 24.9 and 6.3 gm. daily, respectively, and contributed 13.3, 11.5 and 2.9 per cent of the total calories. The diets supplied only about a

TABLE III
Dietary Intake of Black Caribs in Livingston, Guatemala

Nutrients	Average Daily Intake (mg.)	Nutrients	Average Daily Intake (gm.)	Per cent of Calories
Calories	1949	Total protein	53.3	10.9
Vitamins		Animal protein	23.1	4.7
Vitamin A*	1053	Carbohydrates	289.4	59.4
Thiamine	0.79	Total fat	66.6	30.8
Riboflavin	0.39	Animal fat	32.6	15.1
Niacin	8.38	Coconut fat	13.2	6.1
Vitamin C	56	Fatty acids	63.3	29.2
Minerals		Saturated	28.7	13.3
Calcium	220	Unsaturated	31.2	14.4
Phosphorus	790	Monounsaturated	24.9	11.5
Iron	12	Polyunsaturated	6.3	2.9

* International units.

quarter of the recommended allowances for calcium, vitamin A and riboflavin, and slightly less thiamin, niacin and ascorbic acid than recommended.

The clinical examinations revealed few positive findings. Among the 182 persons examined, only one case each of angular stomatitis, cheilitis, abnormally red tongue and xerosis of the skin were noted. Follicular hyperkeratosis, common in most Guatemalan populations, was found in only two cases. The most suggestive positive signs were the occurrence of either hypertrophy or atrophy of the papillae of the tongue in four of the twenty preadolescents, eight of twenty-one adolescents and twenty-four of 141 adults. One preadolescent and five adults had some degree of nasolabial seborrhea.

Although increased vascularization and thickening of the bulbar conjunctiva were noted in a considerable proportion of this group, they were considered to be due to the effects of sun and dust rather than a deficiency. Forty-eight adults were noted to have marginal gingivitis associated with loss of teeth and poor dental hygiene; it was not of the generalized type associated with a dietary deficiency. The general physical appearance of the group was very good. None were classified as undernourished.

COMMENTS

Despite the apparent deficiency of certain vitamins in the diet, the clinical examinations gave the impression that the people in Livingston were well nourished and not suffering from significant deficiencies of specific nutrients. The body measurements also supported this impression. Adult heights were not markedly different from those reported for the populations in the United States[17] and Canada.[18] While mean body weights were lower than those for the United States,[17] few subjects were markedly underweight and some were obese. With the single exception of the group of women age fifty to fifty-nine years, the skinfold measurements were within normal limits for well nourished populations, although they averaged less than the mean values reported in the United States.[17,19]

The serum cholesterol levels among the Black Caribs are much higher than those found in the population of any other low income group thus far studied in Central America, although the general living conditions are not markedly different from those of some populations in which relatively low serum cholesterol values are found.

Since the diet of the Black Caribs in amount and variety of fat and protein approaches, although it does not equal the diet of upper socioeconomic groups in the area, the present data seem to present one more example of cholesterol levels paralleling dietary calorie, fat and protein differences rather than racial, climatic or other non-dietary factors. Even though the diets of the study group appeared to be deficient in calcium, riboflavin and vitamin A, there is no indication that this influenced either physical appearance or cholesterol levels.

It has been previously noted that persons of Negro origin living in this hemisphere have a relatively high frequency of hypertension.[20,21] In Panama, this is particularly striking because they are living alongside a mestizo group with a very low prevalence of hypertension. There has been much speculation as to the relative role of genetic and environmental factors.[21] The present frequency of hypertension in nearly 10 per cent of adults over forty years of age living under conditions apparently identical with some Indian and mestizo populations with a low prevalence of hypertension is suggestive of a genetic susceptibility. As Bays and Scrimshaw[22] have pointed out, however, comparative data of this type are subjected to many fallacies and do not afford conclusive evidence of a genetic effect.

SUMMARY

A nutritional and dietary survey was conducted among the Black Carib population of Livingston. Eighty-eight males and ninety-four females ranging in age from six to eighty-nine years were examined, and blood samples were obtained for blood typing and serum cholesterol determinations. The diets were markedly low in vitamin A, riboflavin and calcium, although upon clinical examination, no evidence of severe nutritional deficiencies

was found. Body height, weight and arm skinfold thickness indicated a relatively well nourished population, although nearly 10 per cent of those over forty years of age had significantly elevated blood pressures. The serum cholesterol levels were found to be considerably higher than those observed among other rural lower income Guatemalans, and paralleled the higher total fat and greater contribution of saturated and monounsaturated fatty acid in their diets. Their serum cholesterol and dietary protein and fat intakes were only slightly lower than those of upper income persons in Guatemala and populations studied in the United States. Serum cholesterol levels observed in the Black Caribs are higher than in other Guatemalans living under similar circumstances. This is believed to be due primarily to dietary differences most probably in fat and proteins rather than to other environmental or social factors.

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Diet Therapy

New Dimensions for Public Health Nutrition

The Challenge of Chronic Disease and Aging

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THE shifting focus and enlarging scope of health programs to meet today's changing health needs offer new challenges to nutritionists as well as other health workers. Since the turn of the century the United States population sixty-five years and older has multiplied four times in contrast to a doubling of the total population. Almost 9 per cent of the total population is now sixty-five years old or over and it is predicted that the proportion will remain constant in the next decade.

In addition to an aging population, we are faced with an unprecedented number of persons with chronic disease, impairment, or long-term illness. Reports from the National Health Survey[1] show that 70 million persons in the United States have one or more chronic conditions; this amounts to 41 per cent of the noninstitutional civilian population.

While chronic illness may occur in persons of all ages it is found with greater frequency among older persons, and about three of every four persons over age sixty-five are afflicted with one or more chronic conditions.

The concomitants of aging and the control of long-term illness are amenable to the public health method and there is evidence of increased concern and activity in chronic disease programs by official and voluntary health agencies. Today there are over thirty administrative units for chronic disease in state health departments compared to three such units in 1945. Visiting nurse associations report that up to 75 per cent of their total visits are made on behalf of patients with long-term illness.

NUTRITION, CHRONIC DISEASE AND AGING

Nutrition is the most important single factor affecting health. This is true at age 1 or 101, but too often this fact is overlooked in the development of new health programs. Nutrition is a specific factor in the prevention and in the control of many chronic diseases.

Weight control is important in the primary prevention and control of certain diseases. Joslin[2] states that obesity is "the most activating factor next to heredity" in the development of diabetes, and he notes that 85 per cent of those persons with diabetes were overweight before the condition was diagnosed. A study of overweight and nonoverweight adults revealed twice as much chronic illness among the overweight group.[3] Persons with diabetes, arthritis, gout, and many forms of heart disease experience better control of these conditions and are more comfortable when they are of normal weight.

The therapy of persons with long-term illness often includes modifications in the kinds and amounts of food eaten. The Commission on Chronic Illness reported[4] that 43 per cent

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TABLE I

Chronic Condition	Rate per 1,000 Population	Source of Data
Overweight (10 per cent or more above "desirable weight" for persons thirty years of age or over)	200.0	National Estimate by Division of Special Health Services, P.H.S.[5]
Arthritis and rheumatism (persons fourteen years of age and over)	63.9	U. S. National Health Survey[6]
Diabetes	16.9	National Estimate by Division of Special Health Services, P.H.S.
Tuberculosis	12.0	National Estimate by Division of Special Health Services, P.H.S.
Ulcers of the stomach or duodenum	14.4	U. S. National Health Survey[7]
Gallbladder disease	8.0	Chronic Illness Survey, Baltimore, Maryland[8]
Diseases of the liver	3.8	Community survey, Hagerstown, Maryland[9]

of the patients in general hospitals with long-term illnesses in one state were on some type of "special diet." Table 1 shows the estimated rate per 1,000 population of persons with some chronic conditions in which diet plays an important therapeutic role.

The economic status of the elderly is a factor that influences the nutrition of this group. Difficulties in obtaining adequate food loom large for three-fifths of the population aged sixty-five years and older who have a cash income of less than $1,000 annually[10] and many of these persons need help in learning which foods to select to obtain the most food value at the least cost.

Psychologic, social and physiologic factors also influence the appetite, food intake and nutrition of the aged.

WHAT IS BEING DONE

The majority of state health agencies employ one or more nutritionists and in most states they give some service to chronic disease programs regardless of their administrative placement. In at least ten state health agencies individual nutritionists are administratively responsible to chronic disease directors or give the major portion of their time to chronic disease activities. This is a beginning. Opportunities for nutritionists in new health programs are unlimited in variety and scope.

Public health nutrition consultation at the Federal level is provided on a categorical basis from the Chronic Disease Program, the Heart Disease Control Program and the Division of Indian Health of the U. S. Public Health Service and from the U. S. Childrens' Bureau. Only the activities of the Chronic Disease and Heart Disease Control Programs will be highlighted in this paper, however. Consultants from these programs are concerned particularly with the nutritional and dietary aspects of heart disease, diabetes, services for the aging, nursing home care and organized home care. They work through the Public Health Service Regional offices located in New York City; Charlottesville, Virginia; Atlanta, Georgia; Chicago, Illinois; Kanasa City, Missouri; Dallas, Texas; Denver, Colorado; and San Francisco, California, to consult with and advise official and voluntary health agencies upon request. Consultation includes such topics as program planning and evaluation, recruitment and resource materials. Frequently program administrators are advised regarding the potential contribution of nutritionists to chronic disease activities and suggestions may be made about desirable qualifications and recruitment possibilities. The consultants also participate in such activities as educational workshops and conferences, and assist in planning and conducting research and study projects.

Recently one of the consultants assisted a state health department in developing plans for the nutritional aspects of a two-day educational program in diabetes. One health department was given assistance in planning and in carrying out a food service study in selected

nursing homes. In another state help was given to the licensing agency regarding an educational program for nursing home administrators. Later the nutritionist contributed to the program by serving as a consultant during the conference.

The development of guide materials for use by the patient and professional worker is also an important contribution. Nutritionists from the Chronic Disease and Heart Disease Control Programs collaborated with professional organizations in the development of the standardized, simplified materials for use by persons with diabetes or who require sodium-restricted diets. The nutritionists have also participated in the preparation of filmstrips, records, and other material for patient and professional diabetes education. They are contributing to the development of recipe booklets for use with sodium-restricted diets and materials for physicians regarding fat- and cholesterol-restricted diets. A visual aid in the form of a flipchart has been developed to assist professional personnel with food service training programs for nursing home personnel. This teaching tool and an accompanying Instructor's Guide have been made available to state health departments.

The nutritionist at the federal, state or local level offers to chronic disease and aging programs her knowledge concerning food habits of the population served, the nutritional needs of specific groups, and how to plan and prepare food to meet these needs, the availability and cost of food in the community. The nutritionist's training in the dietary aspects of the treatment of medical conditions enables her to make contributions to professional and lay persons regarding the care of the chronically ill and aged. Her knowledge of quantity feeding and institutional management is useful in developing programs with nursing homes and other group care facilities. Skills in interviewing and nutrition educational technics enable her to work effectively in all of these newer programs.

Patient Education

Weight control projects by the "group method" have been conducted by a number of public health nutritionists who have joined forces with colleagues in medicine, agricultural extension service, nursing and health education for such ventures. Some states have developed cooperative programs which are continuing. In others, the public health nutritionist has been the catalyst to encourage and assist interested, qualified groups to sponsor group classes.

Persons with diabetes usually need assistance in following the diet plan prescribed by their physicians, and benefit from understanding food values. In several states, nutrition units have offered group diet instruction to such patients. Impetus for such programs and details of carrying them out may vary, but such activities should have the support and approval of organized medicine; they require individual patient referral by the physician. In a midwestern state, nutrition education for patients with diabetes was an outgrowth of diabetes case-finding programs. At the time persons suspected of having diabetes were referred to their physicians for diagnosis and follow-up, the physicians were invited to refer newly diagnosed diabetic patients to group classes conducted by health department personnel.

In an eastern state health department nutritionists conducted classes regularly for persons with diabetes at the request of the local medical society and in cooperation with the local dietetic association. Frequently, and preferably, nutritionists join forces with other professional health workers so that diet instruction is provided as part of an over-all guidance to the diabetic patient.

Public health nutritionists are finding increasing opportunities to work with state and local voluntary associations on behalf of persons who require sodium-restricted diets. Classes similar to those for persons with diabetes have been held in a number of places.

Services to Institutions

One estimate[10] indicates that as a group persons over age sixty-five use two and a half times as much general hospital care as the average for persons under sixty-five years of

age. According to Solon[11] nursing home beds are occupied by persons whose average age is eighty, and 25,000 "homes" provide nursing or supportive services to chronically ill, convalescing, aged, disabled or infirm persons. These facilities, and approximately 3,500 small general hospitals (with bed capacities of less than 100), are focal points for service from public health nutritionists. Most of these institutions, because they are small, employ nonprofessional food service personnel. Administrators and staff need assistance with menu planning, food purchasing, therapeutic diets and other aspects of food service as well as with patient education. Nutritionists participate in training food service workers and offer consultation to administrators in a number of states. They also assist licensing agency personnel in their evaluation of food services in these facilities.

The nutritionist in one city health department assists the nursing home program in a number of ways. When the prospective administrator visits the health department to apply for a license, he meets with the nutritionist as well as other members of the staff. From them he learns the requirements he must meet to comply with the regulations and the services available from the department. With the cooperation of the nursing home association regular group meetings on nutrition and food service are presented for operators of existing homes. The nutritionist in this department, and a number of others from the East Coast to the far west, are responsible for periodic newsletters on food service which are distributed to nursing homes and other small institutions.

In a number of states, public health nutritionists have taken the leadership to develop, or have cooperated in the preparation of, diet manuals. These have been intended primarily to improve the quality of general and therapeutic diets in small institutions, but frequently they have served also to standardize and modernize diet therapy practices.

Assistance to institutions is provided in many other ways such as preparation of food service manuals; distribution of menu planning forms and nutrition guides; help in the selection and use of equipment; suggestions about kitchen layout and work simplification technics.

"Meals on Wheels"

"Meals on Wheels" is the name given to programs which deliver meals regularly to elderly, homebound persons who are unable or disinterested in preparing food for themselves.[12] Based on an idea originated in England, the programs have great public appeal and have been developed in about twenty localities in the United States. Nutritionists can assist in evaluating the community need for this type of program and in working out the details of preparing and serving attractive meals for a modest fee. In one state, nutritionists work with the sponsors to help insure menus of high quality and to assist in evaluating the service periodically. In another program sponsored by a voluntary agency, the nutritionist assumes all administrative details of the food service.

Organized Home Care

The role of the nutritionist in organized home-care programs has been delineated recently.[13] This is an area in which nutrition service has a great potential yet to be developed in most of the sixty programs in operation today. The nutritionist in one county health department gives regular service to an organized home-care program in her community; she not only attends all planning and evaluation conferences and consults with the professional staff on selected cases, but also gives direct service to some patients in their homes.

FUTURE TRENDS

What of the future for public health nutrition in new health programs? It seems inevitable that the amount and intensity of effort on behalf of the well and sick middle-aged and older adult will increase. Hopefully nutrition programs of the future will be geared to give adequate service to everyone throughout the span of life.

One group, which may well receive more emphasis in nutrition education programs, are middle-aged adults. Nutrition should be included in preretirement counseling programs that are becoming popular in industry. In

one series in which this was done the nutrition session had the highest rating in the class evaluation. Also nutrition in its preventive and therapeutic aspects must be included more frequently in the health education programs of industry by such means as providing written materials, by working with industrial nurses and by giving individual nutrition counseling.

Dietary counseling is another field which holds promise for the future. Such counseling helps nonhospitalized patients understand and follow prescribed dietary regimens adapted to their individual needs. The public health nutritionist is in a unique position to see that such service is available. Depending on the local situation she may help to develop a referral system for exchange of pertinent diet information between the hospital and other community agencies, or she may assist in setting up a program to give individual counseling to persons referred by physicians. In any event she can assist public health nurses by providing them with necessary tools and technics to advise patients about diet.

There are about 5.5 million persons in the United States (or 3 per cent of the population) who have some type of chronic mobility limitation.[1] Most of these persons can be restored to levels of self-care. Medical rehabilitation programs are organized to enable chronically ill, disabled or aged patients to maintain or recover functional capacity for the physical, mental and social demands of daily living. This requires the services and skills of all members of the health team. The nutritionist's role in restorative services has barely been touched, although she has much to contribute to professional staff and patients. Unless disabled persons are in a good state of nutrition how can they possibly have stamina to use crutches or to relearn the simple "activities of daily living" that spell the difference between a bare existence and a meaningful life? A nutritional history, taken on each person at the rehabilitation center or hospital, plus follow-up assistance to interpret and assist in making desirable diet changes, is one example of the service the nutritionist can provide.

What about the elderly person who is at home and is unwilling or unable to prepare food for himself? As already stated "Meals on Wheels" represents one method of providing food for such persons. The serving of hot meals at senior citizens' centers is being tried in a few places; one home for the aged offers noon day meals to nonresidents who wish to purchase this service. Other ways to provide food service to oldsters should be studied and nutritionists may need to stimulate such exploration.

SUMMARY

Public health nutrition has specific and valuable contributions to make to programs focused on chronic disease and aging. In this new and challenging field the ways in which nutritionists can function are limitless. A few examples have been given to illustrate some ways nutritionists are serving and may increase services to their professional colleagues and the public in newer public health programs.

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Clinical Reports

Sex Variation in the Nutritional State of Urban Zulu Adults

I. Weight, Skinfold Thickness, and Skin and Mucosal Lesions

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Differences are to be expected in the nutritional state of the two sexes, both for biological reasons and as a result of the occurrence of socially determined differences in the diet and activities of men and women. The present study explores the occurrence of such differences among urban Zulu adults.

The investigation, which formed part of a broader nutrition-hypertension study project, was carried out from October to December 1958 on a random sample of the adult Zulu residents of a housing scheme for Africans in the City of Durban, South Africa. Most of these persons had been born in rural areas, this, like the steady growth of the housing scheme since its establishment in 1934, being symptomatic of the rapid townward movement of Africans which has characterised South Africa's industrial expansion in the current century.

The population of the housing scheme is predominantly Zulu (72.5 per cent). The men are mainly employed in manual occupations. Approximately a fifth of the women are employed away from home, mainly in domestic service. The median income per caput is £2.6.0. (about $6.40) per month, and the median food expenditure per caput £1.5.0. (about $3.40) per month. The main cereal eaten is refined maize, which contributes slightly over 50 per cent of the average caloric intake. The usual diet includes relatively small quantities of green or yellow vegetables, milk, meat and fish.[1] There is a high prevalence of malnutrition in the community. Infection of the respiratory tract and bowel are common, as is intestinal helminthiasis. The infant mortality is high (70 per 1,000 live births in 1958). Aspects of the health and way of life of this community have been discussed more fully elsewhere.[2–4]

METHODS

Nutritional Assessment

Detailed observations were confined to (1) weight and height, (2) arm skinfold thickness, (3) selected skin signs and (4) selected mucosal signs. Each person's skin and mucosal surfaces were examined simultaneously by two of the three participating physicians who reached agreement in respect to each finding. In addition serum protein and cholesterol estimations were carried out, and the occurrence of edema noted; these findings will be reported elsewhere.

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Weight and Height. Our subjects were weighed unclothed. Height was measured with the line of sight horizontal. The relative weight of each subject was assessed by expressing the actual weight as a percentage of the mean weight of a U. S. adult of the corresponding sex, age and height. A relative weight of 125 per cent indicated that the person was 25 per cent overweight, in relation to this standard. The U. S. standards used were derived from the U. S. Medico-Actuarial Mortality Investigation.[5] As our subjects were measured without clothes, the findings were first adjusted by adding 1 inch and 10 pounds (for men) or $1\frac{1}{2}$ inches and 6 pounds (for women). Subjects aged over fifty-five were compared with the standard provided for persons of fifty-five years.

Arm Skinfold Thickness. The measurement was made half-way down the back of the right arm, over the triceps. The site was marked, a fold of skin and subcutaneous tissue lifted, and a Harpenden skinfold caliper[6] applied. Three measurements were made at each site by each clinician, and a mean calculated. If there was much difference between measurements, further observations were made.

Skin Signs. Observations were confined to five skin areas, *viz.*, (1) face, (2) front of trunk, (3) back of trunk, (4) shoulders and upper arms, and (5) thighs. The skin signs sought were those listed subsequently. The intensity of each sign was scored for each of the five skin areas: 0 absent or minimal, 1 mild, 2 moderate, 3 marked. Thus five figures were recorded for each skin sign. The total of these five scores was regarded as the score for the sign, and used as a measure of the grade of the abnormality.

Three abnormalities of the pilosebaceous follicles were recorded:[7] *phrynoderma*, or follicular hyperkeratosis; *dyssebacia and/or folliculosis*, including follicular enlargement (but not frank acne) on the face and in the sternal and interscapular regions; and *follicular enlargement* or "permanent gooseflesh" in skin areas other than those mentioned. Five nonfollicular skin signs were recorded, *viz.*, *dryness*, *dullness*, *increased reticulation* (an accentuation of the normal reticulation of the skin, often with superficial crinkling), *flaking* and *hyperkeratosis*. As there was some intercorrelation among these five signs, the total of their five separate scores was used as an index of "*skin xeroses.*" This combined score was thus an index of skin dryness and dullness, and the commonly associated textural abnormalities.

Mucosal Signs. Observations were confined to the lips, gums and tongue, in which the signs listed subsequently in Table II were sought. The degree of abnormality of each of these three areas was clinically assessed as "none or minimal," "mild," "moderate" or "marked." These ratings were based on the nature, degree and extent of the signs found.

Other Data

Apart from the nutritional assessment, a rapid general physical examination was carried out. This was supplemented, when disease was suspected, by information available in the clinical files of the Institute of Family and Community Health, Durban, and, in a few cases, by chest roentgenography. In addition, urine specimens were examined.

Each subject was interviewed regarding his current dietary habits and other aspects of his current and past life situation.

Each person was asked how many times a week he or she usually consumed meat or fish, eggs, fruit, dried legumes, green or yellow vegetables, "tshwala" (kaffir beer) and spirits. Where a range was supplied in the answer, the mid-point of the range was used. A further series of questions dealt with the consumption of milk, either as a beverage, in other beverages, with porridge or as "amasi" (soured milk). By the use of conversion factors based on an unpublished dietary study of the community,[1] it was possible to express approximate milk consumption in terms of pints per week. The average number of teaspoonsful of sugar consumed per day, in beverages or porridge, was used as an approximate index of sugar consumption.

The diets of persons having 5 or more pints of milk a week, and/or meat or fish daily were rated as "superior," and other diets as "in-

ferior." Diets rated as "superior" were found to be characterised also by a more frequent consumption of eggs, fruit and green or yellow vegetables, and a higher consumption of sugar.

Further questions dealt with household income, food expenditure and household size. The approximate mean household monthly income per caput and food expenditure per caput were calculated for each person. Two social class ratings were used, one based on the occupation of the subject, or her husband in the case of married women, and the other on that of the subject's father. The method of classification was based on type of work, educational attainments, training requirements and income.[8,9]

Rurality was assessed by asking "Where did you live for most of your childhood (to the age of sixteen)?" and "How long have you been living in an urban area?" The answer to this latter question was expressed as a percentage of the subject's age. In the few cases in which a person was uncertain of his or her age, an estimate was made, based on the dates of historical events of which he or she was aware.

SUBJECTS

The sample was comprised of adult Zulu residents, aged eighteen or more, of a randomly selected one in seven sample of all the homes in the housing scheme. The persons examined included 272 women (77 per cent of the initial sample) and 112 men (45 per cent). Eleven per cent of the women, and 31 per cent of the men, could not be examined because they were working and unable or unwilling to attend for examination either during the week or on weekends. An additional 13 per cent of the women, and 22 per cent of the men, refused to be examined.

In view of the high failure rate among the men, it was not certain that the male sample, in particular, was a representative one. Accordingly, visits were made to the homes of half the persons who had not been examined in order to find out whether the male and female samples were representative in respect to age, marital state, parity, social class, income, food expenditure, education and other variables. It was found that in the female sample persons under twenty years of age and over sixty-four years were over-represented. The analysis was therefore confined to persons aged twenty to sixty-four years. Among such persons, the only differences between the examined and nonexamined persons related to education, rurality and social class. The differences in education and rurality were similar in both sexes, and thus did not vitiate a sex comparison of nutritional state. The social class difference, however, applied to men only; men in class v (unskilled labourers) were significantly under-represented among the persons examined. It was subsequently found, however, that among the men social class bore a significant relationship to only one of the nutritional indices used, namely, serum globulin concentration. It was concluded, therefore, that there was no reason to doubt that, except with regard to serum globulin, any differences found between the men and women examined were probably applicable to the Zulu population of the community as a whole.

In view of the known effects of pregnancy on nutrition, twenty-eight women who were pregnant, and thirty-five who were within six months of their last pregnancy, were excluded from the main analysis. Six persons with active or recent tuberculosis, five with congestive cardiac failure, one with diabetes and one with hemiplegia were likewise excluded. The fifty persons in whom minor disorders were diagnosed were not excluded. Thus the findings which follow relate, except when otherwise stated, to ninety-eight men aged twenty to sixty-four years who were not suffering from major diseases, and to 173 women of the same age group, not pregnant or recently pregnant, and not suffering from major diseases.

Complete data were not available for all persons. One man had acute sunburn, and his skin findings were not recorded. Also, occasional questions were inadvertently left unasked in the co se of interviews. When information was lacking, the person concerned was excluded from the relative analyses.

TABLE I
Nutritional Findings in Men and Women

Findings Showing Significant Sex Variation	Men	Women	P
Weight (lb.)			
Mean	141.9	157.6	<0.001
Standard deviation	25.0	34.9	
Median	138.0	155.7	
Relative weight			
Distribution (%)			
Under 90%	20.4	5.8	<0.001
90–109%	55.1	23.7	
110–124%	19.4	29.5	
125% or more	5.1	41.0	
Skinfold thickness (mm.)			
Mean	9.0	22.5	<0.001
Standard deviation	4.5	8.9	
Median	8.0	22.0	
Gum abnormalities			
Per cent rated as "mild" or worse	71.1	52.7	<0.01
Dyssebacia/folliculosis			
Per cent with scores of 3 or more	62.9	30.6	<0.001

Findings Showing No Significant Sex Variation	Men	Women
Phrynoderma (scores)		
Distribution (%)		
0	36.1	41.0
1	30.9	28.9
2–3	24.7	22.0
4+	8.2	8.1
Follicular enlargement (scores)		
Distribution (%)		
0	48.4	57.8
1	35.1	28.9
2–3	14.4	12.7
4+	2.1	0.6
Skin xerosis (scores)		
Distribution (%)		
0–8	13.4	11.0
9–12	42.3	40.5
13–16	30.9	29.5
17+	13.4	19.1
Lip abnormalities		
Per cent rated as "mild" or worse	6.1	7.5
Tongue abnormalities		
Per cent rated as "moderate" or "marked"	21.4	19.7

NOTE: The findings refer to ninety-eight men and 173 women (ninety-seven men in respect to skin findings).

RESULTS

Nutritional Findings

Significant sex differences were found in respect to relative weight, skinfold thickness, gum abnormalities and dyssebacia/folliculosis (Table I). These sex differences were apparent in all age groups, except when otherwise stated. There were no sex differences in respect to the other nutritional findings summarised in Table I. The detailed mucosal findings are shown in Table II. Three of the gum lesions listed, and none of the tongue and lip lesions, showed significant sex differences.

Signs of malnutrition other than those listed were often observed. Frequent findings, for example, were "mosaic skin" on the legs, a "buccal frieze"[10] and conjunctival heaping. One case of classic pellagra was diagnosed.

The women were considerably heavier than the men, although their mean height was lower (61.7 inches and 65 inches, respectively). By comparison with U. S. standards, 41 per cent of the women were overweight by 25 per cent or more, and 5.1 per cent of the men; 20.4 per cent of the men were over 10 per cent underweight, and 5.8 per cent of the women.

The skinfold measurements of the women were considerably greater than those of the men. The mean measurement of the men (9 mm.) was slightly greater than those of Canadian men, which vary from 6.3 to 8.2 mm. in different age groups.[11] The mean measurement of the women (22.5 mm.) was considerably higher than those of Canadian women, which vary from 12.4 to 17.7 mm. in different age groups.

TABLE II
Prevalence (%) of Mucosal Signs, by Sex

Mucosal Signs	Men	Women
Gum signs*		
Marginal gingivitis	71.4	53.2†
Soft and swollen	58.2	39.9†
Pus	57.1	43.4‡
Red	40.8	40.5
Firm, hyperplastic	18.4	9.8
Bleeding	4.1	4.0
Soft (not swollen)	1.0	1.7
Lip signs		
Dryness	52.0	45.7
Angular flaking	22.4	30.1
Sodden angles	3.1	2.9
Redness	3.1	1.7
Cracked angles	2.0	—
Dry angles	—	2.9
Fissured	—	0.6
Tongue signs		
Papillary atrophy	99.0	95.4
Marginal indentation	96.9	96.5
Fissured	83.7	80.9
Redness	78.6	68.8
Papillary enlargement	28.6	30.1
Magenta hue	1.0	0.6

NOTE: Findings refer to ninety-eight men and 173 women.

* One man and three women were edentulous.

† Significantly different from the values for men ($P < 0.01$).

‡ Significantly different from the values for men ($P < 0.05$).

TABLE III
Percentage Prevalence of Dyssebacia/Folliculosis According to Site

Age (yr.)	No.		Prevalence % of Dyssebacia/Folliculosis			
			Face		Trunk	
	Men	Women	Men	Women	Men	Women
20–29	32	49	90.6	93.9	93.8	65.3*
30–39	33	54	97.0	94.4	93.9	59.2*
40–64	33	70	90.9	74.3	87.9	51.4†

* Significantly lower than the figures for the men ($P < 0.01$).
† Significantly lower than the figures for the men ($P < 0.001$).

The weight, height and skinfold findings have been detailed more fully elsewhere.[12]

Abnormal gums were more common among the men than among the women. The specific gum signs which were significantly more common among the men were marginal gingivitis, soft swelling and the presence of pus (Table II). Typically scorbutic gums were not seen.

The women had significantly less dyssebacia/folliculosis than the men. On the trunk (i.e., in the sternal and interscapular areas), this male preponderance was marked at all ages (Table III). On the face, it was apparent only among persons of forty or more years, among whom it fell just short of statistical significance (P = between 0.05 and 0.10).

In order to assess whether or not these nutritional differences represented separate processes, evidence was sought, in each sex, of correlations between the nutritional findings showing sex variation.

The following were the only significant correlations involving the findings discussed:

(1) Among both men and women, there was a significant correlation between their relative weights and their skinfold measurements ($P < 0.001$). It is accepted that skinfold measurements provide a good estimate of the quantity of fat in the body.[13] This association thus indicated that the sex difference in weight and skinfold thickness was largely a reflection of a single factor, *viz.*, a greater degree of adiposity among the women.

(2) Among the men, there was an association between normal gums and a low relative weight. Of the twenty-eight men with no or minimal gum abnormalities, twenty-seven had relative weights of under 90 per cent; and of the sixty-nine men with mild or worse gum abnormalities, fifty-one had relative weights of under 90 per cent ($P < 0.05$). This association was partly due to a common relationship between age and both these variables. However, the association was apparent in each age group, and significant in one, *viz.*, thirty-five to forty-four years ($P < 0.05$).

(3) Dyssebacia/folliculosis was significantly associated, among the women only, with relative weight (Table IV). The women with more dyssebacia/folliculosis tended to have higher relative weights. This association was apparent among both the younger and the older women, and significant among the younger. There was also a significant associa-

TABLE IV

Relationship of Dyssebacia/Folliculosis to Relative Weight (in Women)

	Total Group		Age 20–34 Years		Age 35–64 Years	
Dyssebacia/follicolosis score	0–1	2+	0–1	2+	0–1	2+
No.	66	107	23	58	43	49
% with relative weights of 110% or more	60.6	76.6	56.5	81.0	62.8	71.4
P	<0.05		<0.05		>0.05	

tion, among the women, between dyssebacia/folliculosis and relatively high (normal) serum albumin levels.

Nutritional Findings and Diet

There were a number of dietary differences between the men and the women (Table v). The consumption of "superior" diets was significantly more common among the men. With respect to individual dietary items, the men had a significant tendency to consume more milk and less sugar than the women, and to take fruit and green or yellow vegetables less frequently. More of the men took alcoholic drinks. There were no significant sex differences in respect to meat or fish, eggs and dried legumes.

In each sex, evidence was sought of correlations between the dietary items for which data were available, and relative weight, skinfold thickness, gum abnormalities and dyssebacia/folliculosis. The following were the only significant associations found.

Relative Weight and Skinfold Thickness. Women having "superior" diets tended to have higher relative weights ($P < 0.01$) and skinfold thicknesses ($P < 0.05$). High relative weights and high skinfold thicknesses were associated, among the women, with a greater consumption of vegetables ($P < 0.02$ and < 0.01, respectively) and eggs ($P < 0.01$ and <0.05, respectively). Among the men, high relative weights were associated with a greater consumption of milk ($P < 0.05$), and the nonconsumption of alcohol ($P < 0.05$). Men taking no alcohol tended also to have higher skinfold measurements ($P < 0.05$).

Gum Abnormalities. Gum abnormalities were associated with the infrequent consumption of fruit, this being the only significant relationship found between gum lesions and any food item. Among the women, the prevalence of "abnormal" gums was 61.5 per cent if fruit was consumed on fewer than three days a week, and only 36.2 per cent if fruit was consumed more often ($P < 0.01$). Three individual gum signs were significantly more common when fruit was consumed infrequently, *viz.*, marginal gingivitis ($P < 0.05$), redness ($P < 0.05$) and the presence of pus ($P < 0.02$). Among the men, "abnormal" gums were not significantly more common among those taking fruit infrequently, but one individual gum sign was soft swelling ($P < 0.05$).

Dyssebacia/Folliculosis. One significant relationship was found between the occurrence of this lesion and diet. Among the men, high dyssebacia/folliculosis scores were more common if meat or fish was taken four or more times a week ($P < 0.02$).

TABLE V

Selected Dietary Findings in Men and Women

Dietary Criterion	Men (% of male sample)	Women (% of female sample)	P
"Superior" diet	44.3	28.7	<0.02
Milk: 5 pints or more a week	29.9	17.3	<0.05
Sugar: 15 or more teaspoonsful a day	5.3	21.1	<0.01
Fruit: 1 or more times a week	46.9	80.1	<0.001
Green/yellow vegetables: 1 or more times a week	80.2	94.2	<0.001
Alcoholic beverages taken	69.1	23.3	<0.001
Alcoholic beverages daily	20.6	1.7	<0.001

NOTE: These findings are based on ninety-eight men and 173 women. In respect to some items, data were not available for one or two persons of either sex.

Nutritional Findings and Parity

In order to explore the possibility that the sex differences found were related to the female functions of child-bearing and lactation, evidence was sought of associations between

TABLE VI

Association of Parity with Gum Abnormalities and Dyssebacia/Folliculosis in Two Defined Age Groups

Data	Age 25–34 Years		Age 35–44 Years	
	Parity 0–2	Parity 3+	Parity 0–5	Parity 6+
Gum Abnormalities				
No.	26	32	27	23
Mean age (yr.)	30.2	30.2	39.9	40.8
Median parity	1	4.5	3	7
% with gums rated as "abnormal"	26.9	46.9	48.1	73.9
Dyssebacia/Folliculosis				
No.	26	33	27	24
Mean age (yr.)	30.2	30.2	39.9	40.8
Median parity	1	5	3	7.5
% with dyssebacia/folliculosis scores of 3 or more	50.0	33.3	48.1*	12.5*

* These two values are significantly different ($P < 0.05$).

parity and relative weight and skinfold thickness, gum abnormalities and dyssebacia/folliculosis.

Relative Weight and Skinfold Thickness. These bore no relationship to parity when age was held constant.

Gum Abnormalities. The prevalence of gum abnormalities increased with parity. The prevalence of mild or worse abnormalities among women whose parity was 6 or more was 71.7 per cent, which was significantly greater ($P < 0.05$) than that among women of parity 3 to 5 (49.0%). Among women of parity 0 to 2, the prevalence was 36.7 per cent. This finding was apparently not related to the effect of age, as it remained apparent when age was held constant (Table VI).

There was evidence that women of a greater parity tended to consume fruit less often. However, when age and fruit consumption were both held constant, it was still found that abnormal gums were more prevalent among women of a higher parity. The sub-groups compared were small, and the differences were not significant; they were, however, consistent.

Dyssebacia/Folliculosis. The prevalence of dyssebacia/folliculosis decreased with parity. Among women whose parity was 3 or more, the percentage with dyssebacia/folliculosis scores of 3 or more was 23.9; among women whose parity was under 3, the percentage with such scores was 45 ($P < 0.01$). This association was apparently not related to the effect of age, as it was apparent in separate age groups. Relative weight, which bore a relationship to dyssebacia/folliculosis, did not vary with parity.

Nutritional Findings and Menopause

A comparison was made of the nutritional findings in the seventeen menopausal and the thirty-four nonmenopausal women aged forty to fifty-four years. No significant differences were found in respect to any of the nutritional findings showing sex variation.

Nutritional Findings and Other Background Factors

There were no significant differences between the men and the women examined in respect to their mean age (37.3 years and 37.6 years, respectively), age distribution, income, food expenditure or childhood rurality.

A number of significant differences were found in respect to other aspects of the subjects' background, namely, social class, marital state and rurality. Careful consideration was given to the possibility that the sex differences in nutrition were related to these differences. In no instance, however, was it possible to explain the nutritional differences on the basis of these factors.

COMMENTS

Relative Weight and Skinfold Thickness

For practical purposes it can be accepted that, although many factors may influence caloric intake and utilization, in general "fat comes only from food, and obesity results only from eating more than is required to meet the energy requirements of the body."[14] The two main factors to be considered in relation to the greater adiposity of the women are their diet and their physical activity.

Although a number of significant associations were found between weight or skinfold thickness and dietary features, and although there

was evidence of sex variation in diet, it was not possible to relate the greater adiposity of the women to the specific dietary features for which data were available. No comparative data were available in respect to the caloric consumption of men and women, or to the intake of maize products, fat or other high-calorie foods.

It is likely that a factor which is related to the women's adiposity is the high valuation which tends to be placed (in this community) on female stoutness. The fuller figure is in many cases regarded, not only as intrinsically attractive, but also as substantial evidence of material prosperity and health. This attitude appears to be deeply rooted in Zulu culture. In his description of the Zulu in olden times, Bryant[15] states:

> "Substantial buttocks is the Zulu taste, and with him is an essential to female beauty. No maiden there would wish or dare to diminish her good points with one of those 'reducing' contraptions so beloved of the modern Bright Young Things. To her, every inch of reduction would entail so much off her value in the Beauty Show. . . ."
>
> "The belle [the Zulu] loves is distinctly plump . . . waist broad, with buttocks massive and protruding; legs thick at the thighs and gradually tapering, over well-stuffed calves, to delicate ankles, and two small white-arched feet, serving as fitting pedestals to so enchanting a figure."

Diet apart, the relative inactivity of the women studied may contribute to their adiposity. It is recognised that a maintained low level of physical activity may contribute to the development of obesity.[16,17] There is little doubt that the women are on the whole considerably less active than the men. Only about a fifth of them are gainfully employed away from home, while the great majority of the men are so employed.[3] The women have small houses to tend, and, unlike the rural Zulu women, are not required to fetch water or fuel, and are involved in little horticultural or agricultural activity. The men, on the other hand, are largely engaged in predominantly manual occupations.[3]

It is thus likely that the greater adiposity of the women studied is largely a reflection of a high caloric intake and/or inactivity, relative to the men.

Gum Abnormalities

Gum changes are common manifestations of ascorbic acid deficiency. Gingivitis has been stated to be "undoubtedly the most frequent and reliable early clinical sign of vitamin C deficiency."[18] However, it is far from being a specific lesion of such a deficiency. Notwithstanding this lack of specificity, the association found in this study between gum lesions and the intake of fruit, alone among the food items studied, strongly suggests that in these subjects gum lesions largely reflect an ascorbic acid deficiency. This hypothesis readily explains the greater frequency of gum signs among the men, who eat less fruit than the women. The average diet of this community is not rich in sources of ascorbic acid, and the cooking methods used are such as to cause a considerable wastage of this vitamin. Under such conditions, it may well be that a low consumption of fruit swings the scales towards the development of gum lesions.

It is likely that there are additional factors accounting for the sex differences found. This is indicated by the association found, among the men, between healthy gums and low relative weights. The possible role of factors other than fruit intake in causing the sex difference in gingival lesions is indicated by the further fact that, even when fruit intake was held fairly constant, gum lesions were found to be more common among men than among women. Of the twenty-four men taking fruit three or more times a week, seventeen had abnormal gums; of the fifty-eight women taking fruit three or more times a week, only twenty-one had abnormal gums ($P < 0.01$). It is likely that, for biological or other nondietary reasons, gingival changes are more common in males than in females. It is noteworthy that among rural Zulu schoolchildren aged seven to eleven years, Kark[19] found that girls tended to have slightly healthier gums than boys. In this connection, it is of interest that on the basis of measurements of the intake and serum levels of ascorbic acid among Californians aged over fifty, Morgan et al.[20] concluded, despite the

absence of clear differences in oral lesions, that men have a higher requirement for this vitamin than do women. U. S. population surveys have tended to show higher blood ascorbic levels in females than in males.[21–23]

The association found between gum lesions and greater parity supports the findings of Martin et al.[24] who found that among pregnant women in Tennessee there was a tendency for serum vitamin C levels to be lower, and gingival changes more common, when the parity was high. U. S. studies have shown that during pregnancy there tends to be a drop in the serum vitamin C level unless the intake is high.[24,25] In addition, the serum levels of lactating women tend to be low.[24] In the light of these findings, it is not unlikely that when the intake of this vitamin is low, gestation and lactation may produce, at least temporarily, a deficiency of ascorbic acid. Lactation tends to be protracted in this community, 69 per cent of mothers breast-feeding their infants for periods of one year or more.[26] In view of this fact, it may well be that among these women, successive pregnancies and lactation result in long periods of deficiency of the vitamin, and thus predispose to the development, in the course of time, of gum lesions.

Dyssebacia/Folliculosis

Dyssebacia/folliculosis was less marked among the women. It was not considered likely that this low prevalence was due to their common use of facial cosmetics, which might in fact be expected to have the opposite effect. It has been claimed that the habitual use of certain creams may, under certain conditions, predispose to the development of comedones and acne-like folliculitis.[27,28] The finding that the women, unlike the men, had a higher prevalence of dyssebacia/folliculosis on their faces than their trunks (Table III) supports this hypothesis, as does the finding that only among the older women, who have a lesser tendency to use such preparations, did the prevalence of facial dyssebacia/folliculosis appear to be less than among the men. It thus appeared that the male preponderance in respect to these lesions occurred in spite of, rather than because of, the use of cosmetics.

Dyssebacia is generally regarded as a common manifestation of "ariboflavinosis," i.e., a riboflavin deficiency resulting from either a low dietary intake of this vitamin, or an increased metabolic need.[29] It seems unlikely, however, that the lesions manifested by our subjects and recorded as "dyssebacia/folliculosis" can be regarded as a specific manifestation of riboflavin deficiency. "Ariboflavinosis" commonly manifests itself as a syndrome, including not only dyssebacia, but also a number of lip, tongue and other signs. Evidence was accordingly sought of an association, in our subjects, between dyssebacia/folliculosis and the various lip and tongue signs recorded. A significant association was found with only one such sign, *viz.*, flaking at the angles of the lips. This relationship was found in women only ($P < 0.05$). Among the men, these two signs were inversely (although not significantly) related. Moreover, in both sexes a frank angular stomatitis was extremely rare (Table II). Not only has a high correlation been demonstrated between the prevalence of this latter lesion and mean levels of riboflavin excretion in various groups of men,[30] but there also appears to be a tendency, when riboflavin intake is low, for such a lesion to be far more common than nasolabial seborrhoea.[31]

In the light of these findings, it appears unlikely that the dyssebacia/folliculosis found in our subjects can be related to a specific riboflavin deficiency. It has been held that sebaceous gland disturbances may reflect a general metabolic disturbance, rather than an ariboflavinosis or an avitaminosis.[32] The positive associations found with relative weight, and the frequent intake of meat or fish support this hypothesis.

Other follicular lesions, *viz.*, generalised follicular enlargement and phrynoderma, did not show a male preponderance. This raises the possibility that dyssebacia/folliculosis, which involves the skin areas rich in sebaceous glands, is a condition in which an important role is played by endocrine factors, which are recognised to play a part in acne vulgaris and

TABLE VII

Prevalence (%) of Dyssebacia/Folliculosis in Pregnant Women, Recently Pregnant Women and Other Women (Age Group 20–44 Years)

Data	Pregnant	Recently Pregnant*	Other†
No.	28	35	132
Mean age (yr.)	31.5	31.9	33.2
% with a dyssebacia/folliculosis score of 2 or more	42.9	54.3	68.2

* Within six months of their last pregnancy. Eighty per cent of these women were lactating.

† Twenty-one and a half per cent of these women were lactating.

other sebaceous gland disturbances.[33,34] It is not impossible that its occurrence in our subjects may be related to the aberrant pattern of estrogen metabolism observed in Africans in this country.[35] It may be that the sex difference is largely due to sex differences in sebaceous secretion. It is noteworthy that among Japanese adults, Suzuki[36] found sebaceous secretion was considerably higher among males.

The sex difference in the prevalence of the lesion could not be explained on the basis of the relationships found between it and diet or relative weight. Comparing persons with similar levels of meat or fish intake, it was found that the male preponderance remained. With regard to relative weight, the heavier women tended to have more dyssebacia/folliculosis than the lighter; yet the women, who as a group were heavier than the men, had less dyssebacia/folliculosis than the men.

The inverse relationship with parity indicated that factors connected with gestation or lactation might in some way protect women from the development of this abnormality. Examination of the findings in pregnant women and women within six months of their last pregnancy supported this possibility. Such women had less dyssebacia/folliculosis than other women of a comparable age (Table VII). In the case of pregnant women, the difference was significant ($P < 0.05$). However, although this possible effect of gestation and lactation may partly account for the sex difference found, it cannot completely explain it, as even among nulliparae, there was significantly less dyssebacia/folliculosis ($P < 0.01$) than among men of a comparable age (Table VIII).

TABLE VIII

Dyssebacia/Folliculosis in Nulliparous Women and in Men (Age Group 18–29 Years)

Data	Men	Nulliparous Women
No.	33	22
Mean age (yr.)	25.3	23.0
% with a dyssebacia/folliculosis score of 3 or more	66.7	27.3

The association with parity and pregnancy supports the possibility that hormonal factors play an important role in the pathogenesis of this lesion in this malnourished community, and that endocrine factors, involving the sex hormones in particular, play a part in the production of the sex difference.

SUMMARY

The examination of a population sample of urban Zulu adults in Durban revealed a number of differences between the nutritional states of men and women.

There was considerably more adiposity among the women, manifested both in higher relative weights and in greater skinfold thickness measurements. It was concluded that this finding was probably largely related to their greater caloric intake and/or their lesser degree of activity, relative to the men. A high valuation is placed on female stoutness in this community.

Gingival lesions were more common among the men. There was evidence indicating that this was partly due to a lesser consumption of fruit by the men. There was evidence, also, of a sex difference independent of fruit intake, possibly related to a biological difference in ascorbic acid requirements. Among the women, an association was found between gum abnormalities and parity, probably reflecting the depleting effect of repeated gestation and prolonged lactation.

Dyssebacia/folliculosis was more marked among the men. There were indications

that this was a reflection of endocrine factors involving the sex hormones in particular.

No sex variation was found in respect to phrynoderma, follicular enlargement, skin xerosis, or lip and tongue abnormalities.

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Sex Variation in the Nutritional State of Urban Zulu Adults

II. Serum Protein Levels and Prevalence of Edema

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THIS paper comprises a further report on an investigation into the occurrence of sex differences in the nutritional state of a population sample of Zulu adults residing in a housing scheme for Africans in the city of Durban, South Africa. A previous report has dealt with the findings in respect to weight, skinfold thickness and the mucocutaneous signs of malnutrition.[1] The present report deals with the findings in respect to serum protein levels and the prevalence of edema.

METHODS

With few exceptions, blood was collected by the method of venepuncture described by Hill and Buckle[2] in order to minimise hemolysis. Specimens were submitted for serum protein estimation using the biuret method of Weichselbaum.[3] Unfortunately, the absolute total protein and albumin levels reported were erroneously low because of the use of defective protein standards. Our absolute findings can thus not be compared with those of other investigators. It is not considered, however, that the presence of a consistent bias of this sort affects the validity of comparisons of different categories of persons within the sample. As the reproducibility of results was, however, only moderate,[4] our conclusions can be regarded only as suggestive, rather than as definitive.

Definite pitting on pressure over the lower end of the tibia was taken as evidence of edema.

The methods used for collecting other data, the sample and its representativeness, and the dietary and other characteristics of the persons studied, have been described previously.[1]

The findings which follow relate, except when otherwise stated, to persons aged twenty to sixty-four years, excluding those suffering from major diseases (tuberculosis, congestive cardiac failure, diabetes and hemiplegia) and women who were pregnant or within six months of their last pregnancy. A total of 173 women and ninety-eight men were examined; serum protein estimations were determined in 158 women (91.3 per cent of those studied) and eighty-nine men (90.8 per cent). We have no reason to believe that any but random factors entered into the selection of subjects for these tests.

FINDINGS

Serum Protein Levels

The over-all findings in the two sexes are summarized in Table I. The serum albumin levels of the women tended to be slightly, but significantly, lower than those of the men, and their serum globulin levels slightly, but significantly, higher. There was no significant dif-

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TABLE I
Serum Protein Findings in Men and Women

	Men	Women	P
No.	89	158	
Serum albumin			
Mean and standard deviation (gm. %)	2.87 ± 0.45	2.74 ± 0.34	<0.05
Percentage with levels under 3 gm.%	62.92	78.48	<0.02
Serum globulin			
Mean and standard deviation (gm.%)	3.57 ± 0.59	3.75 ± 0.58	<0.05
Percentage with levels of 3.5 gm.% or more	51.68	67.09	<0.05

ference in total protein levels. As stated, the true serum albumin levels were probably somewhat higher than the figures indicate. These sex differences were observed both among persons aged under forty-five and among older persons, although the difference in albumin levels attained significance only in the former group, and that in globulin levels only in the latter ($P < 0.05$ in each instance).

Apart from an inverse relationship (among the women) between serum albumin levels and the occurrence of dyssebacia/folliculosis, serum protein levels bore no relationship (in either sex) to relative weight, skinfold thickness or the mucocutaneous signs of malnutrition.

As has been described elsewhere[4] there was considerable evidence (in both sexes) of a relationship between serum protein levels and diet. Among the men, low albumin and high globulin levels were significantly related to the consumption of an "inferior" diet, so defined on the basis of a low consumption of milk and meat or fish, and characterized also by a low consumption of eggs, fruit and green or yellow vegetables, and a high consumption of sugar.[1] Among the women, high globulin levels were related to a low intake of meat or fish and green or yellow vegetables, and a high intake of sugar and dried legumes. There was evidence that the association with diet was independent of income, social class, rurality and other features of the subjects' current and past life situation.

There were no differences of note between the serum protein levels of men and women consuming "inferior" diets. Among persons taking "superior" diets (5 or more pints of milk a week and/or meat or fish daily), however, the

TABLE II
Serum Protein Findings in Persons Having a "Superior" Diet (Women Grouped According to Parity)

	Men	Women		
		Parity 0–2	Parity 3–5	Parity 6+
No.	37	16	13	16
Percentage with globulin level of 4 gm. % or more	8.1	12.5	23.1	37.5*
Percentage with albumin level below 3 gm. %	48.6	68.8	69.2	87.5*

* Significantly different from the corresponding values for the men ($P < 0.05$).

sex differences found in the total group remained apparent. Of the thirty-seven such men, 48.6 per cent had albumin levels below 3 gm. per cent, and 8.1 per cent had globulin levels of 4 gm. per cent or more. Among the forty-five women taking "superior" diets, the corresponding percentages were 75.6 and 24.4, respectively. The difference in respect to albumin levels was significant ($P < 0.05$), and that in respect to globulin levels fell just short of significance (P = between 0.05 and 0.10).

Among the women, there was no significant relationship between parity and serum albumin levels. There was, however, a significant relationship between parity and serum globulin levels. Women of a higher parity tended to have higher levels. Of the fifty-five women of parity 0 to 2, 16.4 per cent had globulin levels of 4 gm. per cent or more, compared with 36.9 per cent of the 103 women of parity 3 or more ($P < 0.02$). An association between high parity and high globulin levels was apparent in each age group (eighteen to twenty-four years, twenty-five to thirty-four, thirty-five to forty-four and forty-five to sixty-four), although significant only in the thirty-five to forty-four year age group, in which high globulin levels were more common among women of parity 6 plus than among women of a lower parity. Among the twenty-one nulliparous women aged eighteen to thirty-four years, the prevalence of high globulin values (4 gm. per cent or more) was 19.1 per cent, a similar figure to that for the forty-one men of the same age group (17.1 per cent).

TABLE III

Prevalence Per Cent of Edema in Women, According to Serum Albumin Levels and Relative Weight

Serum Albumin Level (gm. %)*	Relative Weight (%)†	No.	Prevalence of Edema (%)	P
Under 2.5	Any	39	30.8	<0.02
2.5–2.9	Any	85	17.6	
3 or more	Any	34	8.8	
Any	125 or more	71	31.0	<0.02
Any	110–124	51	15.7	
Any	Under 110	51	9.8	
Under 2.5	125 or more	19	42.1 (A)	A compared with B: <0.01
Under 2.5	Under 125	20	25.0	
2.5 or more	125 or more	44	22.7	
2.5 or more	Under 125	75	10.7 (B)	

* For reasons discussed in the text, the serum albumin levels are unduly low.

† Subject's weight expressed as a percentage of U. S. standard weight for sex, age and height.[5]

This association with parity appeared to be independent of diet. The intake of meat or fish and green or yellow vegetables (alone among the food items associated with globulin concentration) varied with parity, but the association between parity and globulin levels remained apparent when the consumption of these items was held constant. Among persons taking a "superior" diet, the proportion of women of parity 0 to 2 who had high globulin levels and low albumin levels was not significantly higher than among the men, but the proportion of women of parity 6 plus who had such protein levels was significantly greater than among the men (Table II).

With one exception, there was no association, in either sex, between serum protein levels and such features of the subjects' background as income per caput, food expenditure per caput, rurality, marital state, social class, paternal social class and education. Among the men, high globulin levels were significantly more common among persons in the lowest social class (class V, unskilled labourers). As has been stated elsewhere,[1] this social class was under-represented in the male sample. However, even in social classes I through IV, high globulin levels were significantly more common among women than among men ($P < 0.02$).

Edema

Edema was more common among the women. It was found in 20.2 per cent of the women and in 10.2 per cent of the men ($P < 0.05$). It was as a rule slight, being confined to the lower part of the legs, and characterized by only slight or moderate pitting. The sex difference was found only among persons aged twenty to forty-four years, among whom the edema prevalence rates were 15.2 per cent for women and 3.8 per cent for men ($P < 0.05$). Among older persons, the rates were 36.6 per cent and 35 per cent, respectively.

The prevalence of edema was related to serum albumin levels, relative weight and arm skinfold thickness, but not to any of the mucocutaneous signs of malnutrition. Among the women, edema was significantly more common when the serum albumin level was relatively low, or when there was marked overweight (Table III). There was a similar relationship to high skinfold thicknesses, i.e., adiposity. Among the women, edema was most prevalent among those who had low serum albumin levels and were also overweight by 25 per cent or more (Table III). This prevalence was significantly higher than that among the women with neither of these characteristics, and higher, although not signifi-

TABLE IV

Prevalence Per cent of Edema in Women Aged Twenty to Forty-four Years, According to Serum Albumin Levels and Relative Weight

Serum Albumin Level* (gm. %)	Relative Weight* (%)	No.	Prevalence of Edema
Under 3.....	125 or over	40	30.0
Under 3.....	Under 125	57	8.8
3 or over.....	Any	26	3.8

* See footnotes to Table III.

cantly so, than among those with only one of these characteristics. Similar trends were seen among the men, but significant relationships could not be demonstrated, possibly because of the paucity of men with high relative weights, high skinfold thicknesses or low albumin levels.

Among the women in the twenty to forty-four year age group (in which the sex difference in edema was apparent), similar relationships to serum albumin levels and relative weight were found (Table IV). Among women who had low serum albumin levels and were also markedly overweight, edema was significantly more common than among those with similar albumin levels who were less heavy ($P<0.02$), or than among those with higher serum albumin levels, irrespective of their weight ($P < 0.05$). Similar relationships were found when serum albumin levels were taken in conjunction with skinfold thicknesses. In addition, among women of this age group there was a direct relationship between edema and skinfold thickness. Of thirty-one women with a skinfold thickness above 30 mm., 25.8 per cent had edema, as compared to 16.7 per cent of forty-two women with a skinfold thickness of 20 to 29 mm. and to 8.5 per cent of fifty-nine women with a skinfold thickness under 20 mm. The difference between the first and third of these groups was significant ($P < 0.05$).

In view of this relationship between edema and relative weight and skinfold thickness, it is noteworthy that, as has been described previously,[1] high relative weights and high skinfold thicknesses were considerably more frequent among the women than among the men.

Microscopic examination of urine specimens from the persons in the study revealed no evidence of renal disease.

COMMENTS

Serum Protein Levels

The women tended to have lower serum albumin levels and higher globulin levels than the men. In view of the relationship found between serum protein levels and diet, it is likely that these sex differences reflect, at least to some extent, sex variations in diet. It is not possible, on the basis of the crude dietary measures used, to pinpoint specific dietary features which may have contributed to the serum protein differences. However, dietary differences between these men and women were found.[1] In particular, significantly fewer men consumed "inferior" diets (as defined herein), the consumption of which was associated with low albumin levels and high globulin levels. It is not improbable that the lower albumin levels and higher globulin levels of the women, relative to the men, may have been a reflection of their greater consumption of such "inferior" diets.

Even among persons having a "superior" diet, however, there was evidence of a sex difference in serum protein levels. It appeared that dietary factors alone did not explain the sex variation and that factors unrelated to diet were operative as well. It is probable that the association found between higher parity and higher globulin levels provides an indication of one such factor. As has already been indicated, this association between parity and globulin levels was apparently not related to dietary differences between the women of higher and lower parity. We can offer no data which may help to explain the association. It is possible that endocrine factors are responsible, or that in these malnourished women, childbearing and lactation comprise a strain which subsequently manifests itself in a raised serum globulin level. From our data, it appears that the sex difference in serum protein levels is largely related to differences in the usual diet of the men and women studied, and also to the effects of childbearing and lactation.

The literature provides some confirmation of the existence of sex differences in serum protein

patterns. Some investigations have shown no such differences; but of those which do indicate a sex variation, the differences seem to be the same as those in our subjects. Data for rural White Tennessee adults, for example,[6] revealed significantly higher globulin levels and lower albumin levels among women; it was found that the intake of protein and of animal protein was significantly lower in these women than in the men. It is noteworthy, however, that Negro women in Tennessee, who also consumed less protein than men, did not have significantly different serum protein patterns from men. Slightly higher serum globulin levels and lower albumin levels were also found in Norwegian nurses and female medical students, compared with levels in male doctors and students.[7] Higher serum globulin levels in females were also found in a small sample of healthy young Connecticut adults,[8] and among persons aged fifteen or more, white or Negro, in North Carolina.[9] In this latter group, females tended to consume less protein than males.[10] Among white (but not Negro) Philadelphia blood donors, women had significantly higher zinc sulphate turbidity values, i.e., higher gamma globulin values, than men.[11]

It was hoped that an examination of the findings in pregnant and recently pregnant women (excluded from the main analysis) might help to clarify the association between parity and protein levels (Table v). Albumin levels tended to be low in the pregnant women, by comparison either with women who were within six months of their last pregnancy, or with other women of the same age group. It is usual to find low albumin levels in pregnancy, with a rise soon after delivery.[12–16]

Globulin levels, too, tended to be lower, both in pregnant and in recently pregnant women, than in other women. This is an unusual finding, as globulin levels are usually found to be unchanged or slightly elevated during pregnancy.[12,14,15] It is possible, however, that a degree of indirect confirmation for this finding may be derived from a study carried out on African expectant mothers in Johannesburg in 1944.[17] This study revealed a mean globulin level of 2.8 gm. per 100 ml. On the assumption that the mean globulin level of nonpregnant

TABLE V

Serum Protein Findings in Pregnant Women, Recently Pregnant Women and Other Women (Age Group Twenty to Forty-four Years)

	Pregnant	Recently Pregnant*	Other
No.	26	25	123
Albumin			
Mean and standard deviation (gm. %)	2.29† ± 0.39	2.75 ± 0.42	2.75 ± 0.34
Percentage with values of under 2.5 gm. %	69.2‡	28.0	21.1
Globulin			
Mean and standard deviation (gm. %)	3.45 ± 0.60	3.37 ± 0.53	3.66§ ± 0.57
Percentage with values of 4 gm. % or more	19.2	12.0	26.8
Mean age (yr.)	27.7	29.7	32.9
Median parity	3	4	3

* Within six months of their last pregnancy: twenty-two of these twenty-five women were known to be lactating.

† Significant lower than the values for recently pregnant and other women ($P < 0.001$ in each instance).

‡ Significantly higher than the values for recently pregnant women ($P < 0.01$) and other women ($P < 0.001$).

§ Significantly higher than the value for recently pregnant women ($P < 0.02$), or for pregnant and recently pregnant women combined ($P < 0.01$).

women was the same as that of selected healthy Witwatersrand mineworkers, *viz.*, 2.8 gm. per 100 ml.,[18] it was concluded that the globulin concentration did not alter in pregnancy. In the light of the sex variation we have discussed, it is not unlikely that the actual figure for nonpregnant women was in fact higher. If this is so, it would appear that these women, too, manifested a drop in their globulin levels during pregnancy.

A hypothesis which may explain this possible drop in globulin levels during pregnancy can be based on the known facts that high gamma globulin levels are usual in African subjects in Durban, as elsewhere,[19,20] and that the concentration of gamma globulin, unlike that of other globulin fractions, tends to drop in pregnancy.[12,15] It may be that in our subjects, who prior to pregnancy have high globulin levels caused partly by a high gamma globulin concentration, the drop in gamma globulins occasioned by pregnancy is sufficient to outweigh any rise in alpha and beta globulins, so that

there is a net drop in the total globulin level. Zinc sulphate turbidity tests were carried out on some of our subjects, and the results supported this hypothesis: however, as the number of pregnant and recently pregnant women subjected to these tests was small, little significance was attached to this finding.

Such speculations cast no light on the association found between parity and high globulin levels, or on the sex differences in serum protein levels. However, they indicate the need for a careful longitudinal study of an adequate sample of malnourished African women, followed through and beyond their pregnancy. Such a study may be of considerable practical importance, in view of the high reproductive wastage among such women, and the recently demonstrated association between abnormal foetal development and abnormal maternal serum protein patterns.[21]

Edema

In the absence of frank renal disease or congestive cardiac failure, it is likely that the edema found was probably nutritional in origin. Edema is a common manifestation of malnutrition among South African adults,[22] although one whose pathogenesis is far from fully understood.[23]

The association of edema with high relative weights and high skinfold measurements is of interest. One possible explanation is that in edematous subjects, the retained extracellular fluid inflates the body weight and skinfold thickness sufficiently to produce such an association. Observations by Keys et al.[24] on a small group of men (average weight 117 pounds) indicated that on the average the weight of the retained fluid amounted to about 9 pounds in occult edema, to about $12^{3}/_{4}$ pounds with edema graded as "1+" and to about $14^{1}/_{4}$ pounds with edema graded as "2+" or "3+." While this possibility cannot be discarded, it seems likely, in view of the extremely high relative weights recorded in many of our subjects, and the mild degree of edema usually found, that the weight of the edema fluid probably accounts to only a slight extent for the association found between edema and relative weight. With regard to skinfold thickness, it was in fact observed that in many of the fatter subjects, the thickness tended to drop with repeated measurements, apparently as an effect of the local pressure of the caliper. However, when this phenomenon was observed, measurements were repeated until constant results were obtained. This probably minimized the possible influence on our observations of local fluid accumulation.

It thus seems not unlikely, particularly in view of the finding suggesting that women with low serum albumin levels are more likely to have edema if they are overweight as well, that adiposity contributed to the development of edema in our subjects. This might be primarily related to local factors, such as the permeability of capillary walls, tissue tension or lymphatic drainage, or to general factors, such as the considerable degree of water retention found in many obese patients.[25,26] This finding may reflect, too, the effect of a high caloric intake on the need for essential nutrients. It is possible that in potentially edematous malnourished persons, an added obesity or a high caloric intake may contribute to the development of edema.

In view of these considerations, it is likely that the higher prevalence of edema noted in our female subjects is a reflection of their lower serum albumin levels, and their considerably greater adiposity than the men, or of the processes producing these manifestations.

It is noteworthy that elsewhere it has been found that females are less subject to nutritional edema than males.[27]

SUMMARY

The examination of a population sample of urban Zulu adults in Durban revealed a tendency for women to have slightly lower serum albumin, and slightly higher serum globulin levels, than men. There was evidence indicating that these differences were largely related to sex variation in diet, and to the effects of childbearing and lactation.

Edema was more common among the women than among the men. There was evidence suggesting that this was associated with their lower serum albumin levels, and their considerably greater adiposity.

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Serum and Liver Cholesterol, Total Lipids and Lipid Phosphorus Levels of Rats under Various Dietary Regimens

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STUDIES made at this university on the effect of different kinds and levels of fats on the excretion of urinary metabolites of rats and later of man focused our interest on what was happening to the serum and liver cholesterol values of the subjects. Of the nutritional factors which may affect the level of cholesterol in blood, the kind and level of dietary fat has been under intensive study in a number of laboratories. Recent studies involving fats alone or in combination with other factors have been reported. Okey and Lyman[1] found that serum cholesterol values of rats fed 10 per cent coconut oil tended to be higher than those of rats fed partly hardened cottonseed oil. Liver cholesterol storage was greatest on the cottonseed oil diets. When Aftergood et al.[2] used 15 per cent lard and 15 per cent cottonseed oil, they found that feeding the lard diets resulted in higher liver and serum cholesterol values when the diet contained cholesterol.

The fatty acid composition of liver cholesterol esters after feeding diets containing different dietary fats has been studied by Mukherjee et al.[3] The addition of cholic acid to diets was used by Hegsted et al.[4] to increase differences in response when cholesterol was added to diets containing various oils. They found that vascular sudanophilia was proportional to the serum cholesterol levels. Cholesterol, cholic acid and hydrogenated coconut oil were used by Nath et al.[5] to develop a diet for use in the study of nutritional factors affecting serum cholesterol.

Presented in this paper are the findings obtained from a study of the effect on the serum and liver cholesterol, total lipids and lipid phosphorus of feeding lard and cottonseed oil at three levels to young rats for two periods of time. Concomitant studies were made on the effect of the addition of lecithin or of saponin to the basal diet.

METHODS

Male and/or female weanling rats of the Sprague-Dawley strain or male rats of the Holtzman strain weighing 45 to 60 gm. were used in these experiments. The tissues from a limited number of older rats were also available for analyses. The rats were housed in individual cages with screen bottoms. They were fed a stock colony diet for one week before they were given the experimental diets.

At the end of the experiment the rats were decapitated, the blood was collected, and the livers removed. Food was removed twelve hours previous to killing from all rats fed cholesterol. Livers and serum were frozen until analyzed for total and free cholesterol, total lipid and lipid phosphorus content.

Serum and livers were analyzed for free and total cholesterol by the method of Galloway et al.,[6] which is a micromodification of the Sperry and Webb method. Total lipid and lipid phosphorus levels were determined by a

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TABLE I
Effect of Sex and Cholesterol in the Diet on Serum Constituents

No. Rats	Cholesterol in Diet (%)	Sex	Cholesterol (mg./100 ml.)		Total Lipid (mg./100 ml.)	Lipid Phosphorus (mg./100 ml.)	C:P Ratio
			Free	Total			
24	0	M	20	92	648	8.2	11
24	...	F	21	92	562	6.7	14
24	1	M	22	148	643	6.5	19
24	...	F	40	286	824	6.4	40
			(±6.0)	(±39.5)	(±13.9)	(±1.3)	(±0.01)

Note: In this and the following tables figures in parentheses represent standard error of mean.

modification of the method of Sperry.[7] The following changes were made for analysis of liver. Whole livers were ground to a smooth consistency with a high speed malt mixer fitted with a special blade. A tissue disintegrator was used to further homogenize a representative sample of the ground liver. Samples of the semi-liquid tissue to equal approximately 0.5 gm. liver were then added to weighed 25 ml. volumetric flasks. Final weight of the tissue was obtained by difference. Approximately 0.4 ml. water was added to produce a more finely divided suspension upon addition of the methanol.

The basal diet contained 20 per cent vitamin test casein, 4 per cent salts and sucrose. The kind and levels of fat and levels of cholesterol, soya lecithin and alfalfa saponin are given with the description of each experiment. The lard was obtained without additives for use in the experimental diets. Cottonseed oil was fed in the form of Wesson oil. All changes in the diet were compensated for by adjusting the level of sucrose. Vitamins were fed at the following levels (milligrams per kilogram) in the diet: thiamine HCl, 4; riboflavin, 4.8; pyridoxine HCl, 2.4; calcium pantothenate, 24; niacin, 48; folic acid, 0.8; vitamin B_{12}, 0.004; biotin, 0.08; p-aminobenzoic acid, 10; ascorbic acid, 100; inositol, 120; choline chloride, 1,000; vitamin K, 0.8; vitamin E, 100; and in I.U., vitamin A, 20,000 and vitamin D, 2,000. Vitamins A, D and E were made up in Wesson oil before being added to the diet.

The rats consuming the low fat diets were allowed to eat *ad libitum*. Rats receiving the two higher levels of each kind of fat were fed isocaloric amounts of diet comparable to the average food intake of rats on the low level of each kind of fat. Adjustments in preparation of diets were made to keep the protein content the same.

RESULTS

In presenting the experimental results no effect is discussed unless it is statistically significant.

Experiment 1

Experiment 1 was designed factorially to study the effects on tissue lipids of feeding diets containing two fats (lard and cottonseed oil) at three levels (5, 13.5 and 20 per cent) with added cholesterol (0 or 1 per cent) for two periods of time (five and eight weeks) to male and female rats. Two replications of the complete design were run with a third replication of the treatments using the medium and high levels of fat which used a total of 128 rats. Average values and significant differences for data calculated for either the two or the three replications were similar.

Weight Gains. As was to be expected since all groups of rats were fed isocaloric amounts of diet, weight gains were similar for 0 or 1 per cent cholesterol and for either kind of fat fed at the three levels. Differences in weight gains were related to sex and to the number of weeks fed. The male rats fed eight weeks made the greatest growth. Liver weights showed a similar picture. Male livers weighed almost twice as much as female livers. Rats

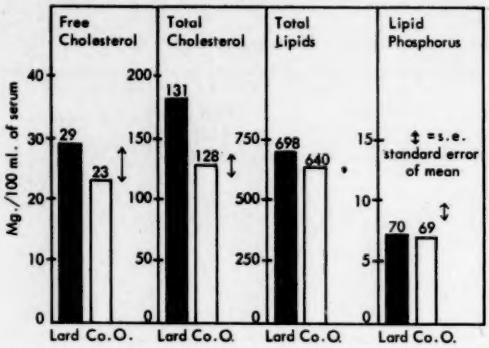

FIG. 1. Effect of lard and cottonseed oil in the diet on serum values.

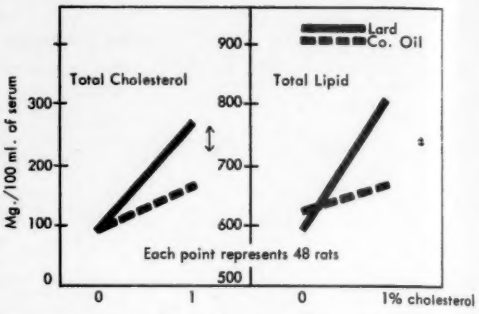

FIG. 2. Effect of kind of fat and cholesterol in the diet on serum values.

fed eight weeks had somewhat heavier livers than those fed five weeks.

Serum Constituents. Feeding of the experimental diets for five or eight weeks did not affect serum lipid values. Female rats had higher free and total cholesterol values than those of the male rats while the male rats had higher values for lipid phosphorus (Table I). The feeding of 1 per cent cholesterol increased the values for free and total cholesterol and total lipids and decreased the lipid phosphorus values. The ratio of total cholesterol to lipid phosphorus was increased. The interaction of sex by amount of cholesterol fed showed that greater differences occurred in the serum data for the female rats than for the male rats as a result of feeding 0 versus 1 per cent cholesterol. Cholesterol values in female rats were approximately twice as high as those in the male rats.

The feeding of lard as compared to feeding cottonseed oil (Fig. 1) resulted in higher free and total serum cholesterol values. These differences occurred only when cholesterol was also fed with the fat (36 versus 25, free and 270 versus 164 mg. total cholesterol per 100 ml. serum). The interaction of kind of fat by cholesterol was significant. Changes in serum lipid values were greater as a result of feeding 0 versus 1 per cent cholesterol with lard as the fat than when cottonseed oil was fed (Fig. 2). The rise in total lipids in the serum was almost entirely due to the increase in total cholesterol. A higher ratio of cholesterol to lipid phosphorus ($P < 0.05$) was shown by the rats fed lard.

Total cholesterol was the only serum constituent influenced by the level of fat fed. The 13.5 per cent level showed the high value of 170 as compared to 158 and 135 for the 5 and 20 per cent levels of feeding, respectively.

Liver Constituents. Increasing the feeding time of the experimental diets from five to eight weeks resulted in higher liver values for total cholesterol, total lipids and lipid phosphorus ($P < 0.01$). Liver values of male rats when compared to those of the female rats were lower for free cholesterol and higher for total lipids (Table II). The other con-

TABLE II
Effect of Sex on Liver Constituents

Sex	No. Rats	Cholesterol (mg./gm.)		Total Lipid (mg./gm.)	Lipid Phosphorus (mg./gm.)	C:P Ratio
		Free	Total			
M	48	2.96	21.7	103	1.14	4.3
F	48	3.30	20.2	92	1.17	4.3
		(±0.22)	(±3.6)	(±12)	(±0.12)	(±0.12)

TABLE III
Effect of Kind and Level of Fat and Feeding of Cholesterol on Liver Constituents

No. Rats	Cholesterol Fed (%)	Kind of Fat	Level of Fat (%)	Cholesterol			Total Lipid (mg./gm.)	Lipid Phosphorous (mg./gm.)
				Free (mg./gm.)	Total (mg./gm.)	Free in Total (%)		
8	0	Lard	5.0	1.66	2.5	66	50	1.10
8	0	Lard	13.5	1.82	2.8	65	55	1.25
8	0	Lard	20.0	1.94	2.8	68	55	0.98
8	0	Cottonseed	5.0	1.88	3.2	59	58	1.25
8	0	oil	13.5	1.69	3.1	54	58	1.25
8	0		20.0	1.87	3.3	57	65	1.17
8	1	Lard	5.0	3.01	27.1	11	100	1.20
8	1	Lard	13.5	2.93	39.9	7	133	1.03
8	1	Lard	20.0	3.40	43.3	8	128	1.03
8	1	Cottonseed	5.0	2.98	25.7	12	106	1.17
8	1	oil	13.5	3.48	50.4	7	174	1.17
8	1		20.0	3.37 (±0.22)	46.4 (±3.6)	7	183 (±12)	1.25 (±0.12)

stituents were not significantly influenced by sex.

When 1 per cent cholesterol was fed in the diet, the free cholesterol was increased by three-fourths while total cholesterol was increased twelvefold. Total lipids were also more than doubled. Approximately half of the rise in liver total lipids was caused by the increase in total cholesterol while in the serum nearly all of the increase in total lipids was made up of cholesterol. Liver lipid phosphorus values were not influenced by cholesterol feeding. Since the total cholesterol rose sharply and the lipid phosphorus did not, the C:P ratio showed a marked rise (2 versus 33 for the 0 and 1 per cent cholesterol feeding).

Total cholesterol, lipid and lipid phosphorus liver values were higher for the rats fed cottonseed oil than for those fed lard (Table III). These findings are in contrast to the trend in the opposite direction shown by the serum data where lard plus cholesterol gave the highest values. The level of fat in the diet significantly influenced the concentration of liver lipids. An increase of one-third to twofold in total cholesterol and total lipid values occurred with an increase in the level of fat from 5 to 13.5 per cent when 1 per cent cholesterol was fed (Table III). Further differences following an increase in fat to 20 per cent were slight. This was true for either the lard or cottonseed oil diets. When no cholesterol was fed, the level of fat in the diet had slight effect on liver values.

Of the total liver cholesterol 7 per cent was in the free state for either the lard or cottonseed oil diets which contained 1 per cent cholesterol on the two higher levels of fat. This value was approximately half the proportion found when no cholesterol was fed. Serum data showed the same trend but the differences in percentage of free cholesterol were of a smaller magnitude. Hence, the bulk of the increase in total liver or serum cholesterol was found in the esterified fraction even though free cholesterol values for liver had doubled in some groups. This finding agrees with that of Okey and Lyman.[8]

It was of interest to compare these results with those from a limited number of older rats. The tissues of twenty-nine adult female rats (ten to sixteen months of age) that had been maintained on diets containing 20 per cent for one or two generations became available for cholesterol analyses. Results with the five fats are shown in Table IV. In these older female rats the lard diets resulted in the highest values for free and total cholesterol. The range of values of total cholesterol was 111 to 163 mg. as compared to 90 and 92 mg.

TABLE IV
Effect of 20 Per Cent Fat in the Diet on Serum and Liver Cholesterol Values of Older Female Rats

No. Rats	Fat in Diets	Serum Cholesterol			Liver Cholesterol		
		Free (mg./100 ml.)	Total (mg./100 ml.)	Free in Total (%)	Free (mg./gm.)	Total (mg./gm.)	Free in Total (%)
8	Butter	28 ± 2.0	136 ± 9.1	20	2.0 ± 0.07	2.7 ± 0.12	73
5	Lard	33 ± 6.6	163 ± 23.3	19	1.9 ± 0.08	2.4 ± 0.05	80
8	Margarine	19 ± 1.4	111 ± 6.9	17	2.0 ± 0.05	2.4 ± 0.10	82
2	Soya Oil	20 ± 2.2	125 ± 0.2	16	1.7 ± 0.35	2.6 ± 0.11	65
3	Wesson oil	24 ± 2.4	137 ± 10.0	18	1.7 ± 0.16	2.3 ± 0.13	73
3	Stock diet	22 ± 2.6	121 ± 18.6	19	2.2 ± 0.23	3.3 ± 1.03	67

for the younger female rats. Variations in liver cholesterol levels between fats were small with the rats on the stock diet having the highest concentration. Liver cholesterol values were similar for the older and younger groups. The over-all age effect appears to be toward higher serum cholesterol values.

Experiment 2

The effect of adding soya lecithin to the diet was studied in this experiment. The levels of lecithin and lard used in the diets are shown in Table v. Five or six male rats were fed each diet for a four week test period.

Weight Gains. The feeding of 20 per cent lecithin without addition of cholesterol resulted in a marked decrease in weight gain (118 gm. gain compared to 141 to 154 gm. for the other lecithin-fed groups and to 140 to 1666 mg. for the lard-fed groups). Rats consuming the 20 per cent level of lecithin had some diarrhea. This may be a partial explanation for the low storage of liver cholesterol and small weight gain. Gains by the other groups did not vary consistently with the treatments. Liver weight was slightly higher for the group fed 20 per cent lard plus cholesterol (11.8 gm. compared to 8 to 9.8 gm. for the other groups fed lard and to 8.2 to 9.4 gm. for the groups fed lecithin). Variation in liver weight was not consistent with the treatments.

Serum Constituents. Serum free and total cholesterol were significantly reduced by the feeding of lecithin as compared to feeding lard when the diets contained 1 per cent cholesterol (Table v). Most of the increase in total cholesterol in the serum occurred in the esterified fraction as changes in per cent of the free fraction were small. Feeding 5 per cent lecithin plus 8 per cent lard gave results similar to the feeding of 5 per cent lecithin with no other fat. All three levels of lecithin kept the serum cholesterol values approximately the same and C:P ratios within normal range even when cholesterol was fed. When lard and cholesterol were fed, serum cholesterol levels rose and C:P ratios were highly elevated. The degree to which the serum cholesterol level rose was not related to level of lard fed. Serum total lipids and lipid phosphorus values were not elevated by the feeding of lecithin alone.

Liver Constituents. In the liver the free and total cholesterol, total lipids and C:P ratio were significantly reduced by the feeding of lecithin instead of lard. The groups fed 5, 13 and 20 per cent lecithin plus 1 per cent cholesterol stored 6, 4 and 3 mg. total cholesterol per gram of liver, respectively, as compared to 27, 23 and 34 mg. for the groups fed the three levels of lard and cholesterol. As was found in the serum, liver values for the groups fed 5 per cent lecithin plus 8 per cent lard were similar to those for the groups fed only 5 per cent for the fat.

Experiment 3

The effect of adding alfalfa saponin to a diet containing 20 per cent lard was studied in experiment 3. The levels of saponin and

TABLE V
Cholesterol Values for Serum and Liver of Rats Fed Lecithin*

In Diet (%)			Serum Cholesterol			Liver Cholesterol			Total Lipids (mg./gm.)
Cholesterol	Lecithin	Lard	Free (mg./100 ml.)	Total (mg./100 ml.)	Free in Total (%)	Free (mg./gm.)	Total (mg./gm.)	Free in Total (%)	
0	5	0	16	86	19	1.97	2.39	82	45
0	13	0	13	81	16	2.14	2.66	80	52
0	20	0	16	94	17	2.19	2.66	82	48
1	5	0	14	96	15	2.44	6.23	39	54
1	13	0	12	84	14	2.27	3.61	63	58
1	20	0	13	84	16	2.23	2.98	75	61
0	0	5	15	89	17	2.46	3.16	78	51
0	0	13	16	104	15	2.39	4.05	59	54
0	0	20	19	111	17	2.18	3.71	59	58
1	0	5	28	207	14	3.32	26.80	12	104
1	0	13	19	126	15	3.05	23.26	13	106
1	0	20	16	136	12	3.12	34.21	9	140
0	5	8	15	90	17	1.96	2.38	82	49
1	5	8	15 (±2)	92 (±9)	16	2.44 (±0.23)	4.32 (±2.83)	56	59 (±10)

* Only the values that were significantly affected by lecithin feeding are included in this table. Six rats were in each group fed lecithin and five in the groups fed lard.

cholesterol used in the diets are shown in Table VI. Two groups of rats were fed 10 per cent alfalfa meal of a known saponin content. Experimental diets were fed for four weeks.

Gains in weight were significantly less for the group of rats fed saponin than for the other groups. When cholesterol was also fed, the gain in weight was somewhat better but not equal to those fed no saponin. Serum total cholesterol values were decreased when 0.5 per cent saponin and 0.5 per cent cholesterol were both fed.

Liver total cholesterol values of rats fed both saponin and cholesterol showed only a two to threefold increase above values for rats fed neither of these compounds as compared to an eightfold increase when cholesterol was fed without saponin. The highest percentage of free cholesterol occurred when only saponin was fed (Table VI). Total lipids were also reduced when saponin was fed alone or with cholesterol. Alfalfa meal fed at a level of 10 per cent of the diet was not effective in preventing a rise of cholesterol values with cholesterol feeding. The saponin intake on this level equaled 0.02 per cent of the diet. Feeding of this low level of saponin to another group of rats was also ineffective and gave values similar to those obtained with the alfalfa meal diets.

COMMENTS

Lard and cottonseed oil were chosen for study as representing saturated and unsaturated fats commonly used in human diets. Feeding these two fats at three levels was used to cover the range of a relatively low intake to that of a high intake of fat. *Serum* cholesterol and lipid values were higher in the groups fed lard plus 1 per cent cholesterol in the present study. However, the *liver* lipid values under the same conditions were higher in the groups fed cottonseed oil except on the low level of fat which gave similar values. Higher liver lipid values with cottonseed oil feeding occurred for either the four or eight week feeding period. Aftergood et al.[2] found that feeding 15 per cent lard and cholesterol for six weeks or longer resulted in higher plasma and liver cholesterol concentrations as compared to findings when cottonseed oil was fed. Differences also occurred after a twelve week feeding period when cholesterol was not added to their fat diets. In the present study, liver cholesterol values for the older rats, which

TABLE VI
Cholesterol Values for Serum and Liver of Rats Fed 20 Per Cent Lard Plus Saponin*

In Diet (%)		Gain in Weight (gm.)	Serum Cholesterol		Liver				
					Cholesterol			Total Lipids (mg./gm.)	Lipid Phosphorous (mg./gm.)
Cholesterol	Saponin		Free (mg./100 ml.)	Total (mg./100 ml.)	Free (mg./gm.)	Total (mg./gm.)	Free in Total (%)		
0	0	149	19	107	2.45	4.08	60	57	1.16
0.5	0	153	20	137	3.38	32.21	10	131	1.12
0	0.5	115	16	102	2.22	3.02	74	47	1.17
0.5	0.5	113	18	100	2.77	10.86	26	80	1.23
0	10 a.m.†	139	17	103	2.56	4.51	57	69	1.28
0.5	10 a.m.	137 (±6.6)	29 (±19)	148 (±8.0)	3.39 (±0.12)	35.46 (±1.79)	10	106 (±12)	1.14 (±0.4)

* Each group contained nine rats.
† a.m. = alfalfa meal.

had been fed a 20 per cent fat diet without added cholesterol for months, were similar for either the lard or cottonseed oil diets. It is of interest that the rats maintained on the stock diet showed the highest liver cholesterol concentrations. Serum cholesterol values for these older rats were highest with the lard diet and next to lowest with the pellet diet. In these older rats, as in the younger rats, the serum values in response to kind of fat fed did not seem to vary in the same way that the liver values did.

Lecithin and alfalfa saponin when fed with cholesterol prevented the rise in serum and other lipids which occurs when lard and cholesterol are fed. When Forbes and Petterson[9] fed roosters diets containing 10 per cent soy bean lecithin, serum cholesterols remained low. The choline in lecithin would probably account for much of its effect in preventing the rise in lipid concentration. Rideout et al.[10] have shown that choline will prevent the large deposition of liver cholesterol associated with cholesterol feeding. The small percentage of plant sterols found in the lecithin might also act to decrease absorption of cholesterol. Swell et al.[11] and others have shown that plant sterols inhibit the absorption of cholesterol. The action of the alfalfa saponin in lowering cholesterol levels could be by this mechanism. Preliminary work in this laboratory showed that 0.5 per cent saponin was not nearly as effective if 1 per cent cholesterol was fed instead of the 0.5 per cent.

The level of fat in the diet had little influence on the concentration of the serum lipids studied whether or not cholesterol was fed. This finding agrees with Nath et al.[5] who found little effect on serum cholesterol when the level of fat was varied. However, it does not agree with Anderson et al.,[12] as well as others in studies with human subjects, who found lower serum cholesterol levels on low fat diets (9 to 16 per cent of the calories). Liver lipid levels showed a sharp rise between the 5 and 13.5 per cent levels of fat fed when 1 per cent cholesterol was included in the diet. Feeding the 20 per cent level of fat further increased the lipid values only slightly.

Elevated serum cholesterol:phospholipid ratios associated with development of coronary lesions in chicks have been reported by Pick et al.,[13] In the present study, female rats had higher serum C:P ratios (lipid phosphorus used as a measure of the phospholipid) and higher serum cholesterol values than did male rats. Serum C:P ratios and cholesterol values were higher for the rats fed lard than for the rats fed cottonseed oil. Feeding 1 per cent cholesterol resulted in an increase in liver C:P ratio. Level of fat did not influence C:P ratio.

Feeding lecithin at all three levels prevented

the serum C:P ratios from being elevated for the rats receiving 1 per cent cholesterol. Liver C:P ratios were also low on the 13 and 20 per cent levels of lecithin and only slightly higher on the 5 per cent level in contrast to the highly elevated C:P ratios for lard plus cholesterol feeding. Serum and liver C:P ratios were similarly low when 0.5 per cent saponin and 0.5 per cent cholesterol were fed.

The C:P ratios for the serum and liver of rats fed 20 per cent lard for 0.5 per cent cholesterol were nearly as high as when 1 per cent cholesterol was fed.

SUMMARY

Young rats were fed isocaloric diets containing lard or cottonseed oil at three levels, with and without cholesterol. Other groups of rats were fed lecithin at three levels and alfalfa saponin.

Rats fed lard and cholesterol had higher serum cholesterol values than those fed cottonseed oil. The reverse picture occurred in the liver with higher values for cholesterol and lipids when cottonseed oil was fed. Results from a small group of older rats maintained on diets with 20 per cent fat for months showed that serum cholesterol was highest on the lard diet but variation in liver cholesterol between fats was small.

Level of fat had slight affect on the serum cholesterol or lipid content. On cholesterol-rich diets, the liver showed a sharp increase in concentration of lipids between the 5 and 13.5 per cent fat diets. Further increases were slight when the diet contained 20 per cent fat.

Cholesterol:lipid phosphorus ratio of the serum was elevated with cholesterol feeding: female rats showed higher ratios than male rats; higher ratios were shown by the rats fed lard as compared to those fed cottonseed oil; and lecithin and saponin feeding prevented this elevation of C:P ratio.

Lecithin at the higher levels and saponin prevented the rise in serum and liver lipids that accompanies feeding of lard and cholesterol.

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ASCN News

Program

AMERICAN SOCIETY FOR CLINICAL NUTRITION

Clinical Section of American Institute of Nutritution

April 29, 1961—1:00 P.M.

Hotel Chalfonte, Atlantic City, N. J.

1. Inhibition of Human Antibody Formation by Vitamin Deficiencies. ROBERT E. HODGES and WILLIAM B. BEAN, *Iowa City*

2. The Effect of Vitamin B_6 Deficiency Induced by Desoxypyridoxine on Whole Blood Transaminase Activity in Human Beings. J. J. WILL, W. T. REPASKY, J. F. MUELLER and H. S. GLAZER, *Cincinnati*

3. A Mechanism of Adrenal Steroid Action—Sulfur Diuresis. GEORGE V. MANN, *Nashville*

4. The Effects of Gastrectomy on Intestinal Absorption. A. B. FRENCH, H. MARVIN POLLARD, A. I. RAGINS and J. T. RATNER (introduced by WILLIAM D. ROBINSON), *Ann Arbor*

5. Folic Acid Deficiency in Alcoholic Cirrhosis VICTOR HERBERT and RALPH ZALUSKY, *Boston*

6. Blood Lipids in Acute Starvation in Humans. JOHN F. MUELLER and JACK M. IACONO, *Cincinnati*

7. Metabolism of C^{14} Palmitic and Linoleic Acids in Normal Human Subjects. LAURANCE W. KINSELL, GEOFFREY WALKER and GEORGE D. MICHAELS, *Oakland*

8. Studies of the Mechanism and Specificity of the Hypocholesteremic Action of Low Protein Diets in Man. ROBERT E. OLSON, SEIJI ITO, K. TRIPATHY and JUANITA EAGLES, *Pittsburgh*

ABSTRACTS OF PAPERS

Inhibition of Human Antibody Formation by Vitamin Deficiencies. *Robert E. Hodges and William B. Bean.* Department of Medicine, State University of Iowa Medical School, Iowa City, Iowa.

Axelrod has shown repeatedly that a deficiency of certain vitamins (particularly pantothenic acid and pyridoxine) will inhibit antibody production in animals. In human subjects we have described the clinical and biochemical effects of feeding diets deficient in these two vitamins with antivitamins added. This report describes an extension of this work.

Five men were fed a formula adequate in all respects except for a deficiency of pyridoxine and pantothenic acid. In addition we gave the antagonists, omega methyl pantothenic acid (4 gm. per day) and desoxypyridoxine (0.4 gm. per day) in divided doses orally. The men became ill after two to three weeks. Immunizations with tetanus and

typhoid antigens were given twice during the deficient period at the third and fourth weeks. After the fifth week the antagonists were discontinued and vitamins were restored to the diets. After recovery two more injections of the serum antigens were given.

All the men had detectable concentrations of tetanus and typhoid antibodies in their serum. They had had previous immunizations during military service. There was no increase in titer of either tetanus or typhoid antibodies after the two immunizations given during the period of vitamin deficiency At the same time levels of gamma globulin in their serum significantly declined.

After restoration of pantothenic acid and pyridoxine to the diets and removal of the antagonists, the titer of gamma globulin increased. The antigenic responses to the second set of injections of tetanus and typhoid antigens were excellent.

These studies indicate that a deficiency of pantothenic acid and pyridoxine induced by a deficient diet and by the antagonists of these two vitamins impaired the antigenic response of five healthy men.

The Effect of Vitamin B_6 Deficiency Induced by Desoxypyridoxine on Whole Blood Transaminase Activity in Human Beings. *J. J. Will, W. T. Repasky, J. F. Mueller and H. S. Glazer.* Department of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio.

Pyridoxine deficiency was induced by giving 4-desoxypyridoxine to ten subjects with a variety of chronic diseases. After a deficiency state developed they were treated with vitamin B_6. Assays of whole blood glutamic oxalacetic and glutamic pyruvic transaminase activity were made during control, depletion, and repletion periods comprising fourteen weeks of observation. Coenzyme saturation of the enzyme was measured, comparing transaminase activity of a blood hemolysate with the activity of the hemolysate following *in vitro* incubation with pyridoxal phosphate. Manifestations of vitamin B_6 deficiency were correlated with transaminase activity. Ten other patients were given vitamin B_6, 12.5 to 150 mg. daily for four to eight weeks, and determinations of transaminase activity were made.

All patients in the deficiency group excreted increased quantities of xanthurenic acid after tryptophane loading, and seborrhea developed in eight. GOT activity and coenzyme saturation progressively diminished in nine subjects. The group mean value of GOT activity was 25.5 units with 63 per cent saturation during the control period compared with 17.6 units with 35 per cent saturation at maximum depletion. Enzyme activity was restored completely by incubation with pyridoxal phosphate. GOT activity was decreased at the onset of seborrhea, but the level of activity did not correlate with the extent or severity of dermatitis. GPT activity and coenzyme saturation were unchanged during depletion. The administration of pyridoxine to the patients who had received desoxypyridoxine and to those who had not, produced a prompt rise in GOT and GPT activity to levels much higher than those of the control period.

GOT activity is a more sensitive indicator of vitamin B_6 deficiency induced by desoxypyridoxine than is GPT. The diminished activity is a result of decreased coenzyme saturation of the enzyme and is restored by pyridoxal phosphate. Supplementary pyridoxine induces a rise in GOT and GPT activity of depleted and nondepleted patients. A rise in transaminase activity following the administration of pyridoxine cannot be considered as proof of vitamin B_6 deficiency.

A Mechanism of Adrenal Steroid Action—Sulfur Diuresis. *George V. Mann.* Department of Biochemistry, Vanderbilt University Medical School, Nashville, Tennessee.

During studies of diabetes and hypercholesteremia induced by adrenal steroids it has been shown that large amounts of sulfur appear in the urine after treatment of animals with 11-dehydro-17-hydroxycorticosterone. This sulfur diuresis is largely comprised of sulfates. It causes a profound negative sulfur balance. The other usual consequences of adrenal steroid therapy were also observed; i.e., uricosuria, loss of carbohydrate tolerance, negative nitrogen balance, hypercholesteremia, suppressed inflammatory response and, in the rabbit especially, suppression of hair growth. These actions may be a consequence of diversion and deprivation of body cysteine and its precursors. The uricosuria is unexplained. The negative nitrogen balance reflects a relative amino acid deficiency. The suppression of inflammation results from the lack of cysteine for fibrinogenesis, the hypercholesteremia is a result of cysteine deficiency for the conversion of cholesterol to bile acid, and hair growth ceases for lack of sulfhydryl compounds. Carbohydrate intolerance reflects a deficiency of insulin which requires cysteine for synthesis. Posterior cortical cataracts in these animals treated with adrenal steroids, as in human subjects, reflects a depletion of lenticular sulfhydryls.

These effects of the steroid can be prevented or

reversed with thiazolidine 4-carboxylate given orally. These findings seem to establish an explanation for many of the physiological actions of adrenal steroids and support this general theory: Aging attributes in mammals are a consequence of cysteine deficiency.

The Effects of Gastrectomy on Intestinal Absorption. *A. B. French, H. Marvin Pollard, A. I. Ragins and J. T. Ratner* (introduced by William D. Robinson). Gastrointestinal Section, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan.

To measure the effect of gastrectomy on intestinal absorption, tests were performed on seventy-seven patients as soon as they were eating well after surgery. All ward patients operated on during the period of the study who agreed to the study were included. Fecal fat in gram per day and per cent of fat intake, fecal nitrogen, glucose tolerance, vitamin A tolerance, I^{131} triolein in stool, urine and blood, Co^{60} vitamin B_{12} and Fe^{59} were used as indices of absorption.

Fecal fat exceeded normal (over 5 gm. per day) in more than two-thirds of the patients after all types of gastrectomy. However, after fourteen Billroth I gastroduodenostomies fat losses did not exceed 11 gm. daily. After forty-one Billroth II resections with gastrojejunostomy half the patients lost more than 9 gm. fat per day and 10 per cent lost more than 15 gm. per day. After proximal and total gastrectomy high fecal fat losses were more common than after 50 to 70 per cent distal gastrectomy, but the maximum losses were no greater. Preoperative disease also influenced postoperative fat losses. Almost 20 per cent of twenty-two patients with duodenal ulcer lost more than 14 gm. fat daily after Billroth II gastrectomy while none of ten patients with gastric ulcer subjected to the same operation lost more than 13 gm. per day.

Fecal nitrogen losses did not reach abnormal levels as frequently as did fecal fat losses. However, fecal fat:nitrogen ratios did not differ significantly from those found in primary and other secondary types of malabsorption. Both fat and nitrogen losses tended to remain constant with the passage of many months.

I^{131} triolein in blood, urine and feces, and vitamin A in blood after a test meal did not consistently reflect fecal fat and nitrogen losses. This was found after all types of gastrectomy. Failure of these tests to reflect steatorrhea did not correlate with the presence or absence of dumping symptoms.

Folic Acid Deficiency in Alcoholic Cirrhosis. *Victor Herbert and Ralph Zalusky.* Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts.

Seventy patients with alcoholic cirrhosis were evaluated. Average erythrocyte mean corpuscular volume was 100 (range: 65 to 128) reflecting a general macrocytosis; average mean corpuscular hemoglobin concentration was 30 (range: 26 to 35) reflecting frequent hypochromia. Average serum vitamin B_{12} level by Euglena gracilis assay was high (>802 $\mu\mu$g. per ml., range: 107 to 2,912). Serum folic acid activity measured by Lactobacillus casei was normal (7 mμg. per ml. or more) in only five, diagnostically indeterminate (between 5 and 6.9 mμg. per ml.) in nine, strongly suggestive of folic acid deficiency (between 3 and 4.9 mμg. per ml.) in twenty-seven, and indicative of folic acid deficiency (<3 mμg. per ml.) in twenty-nine. Urinary formiminoglutamic acid (FiGlu) excretion after histidine loading with 5 to 20 gm. was studied in six subjects with serum folic acid activity (<5 mμg. per ml.) and found to be elevated.

These findings suggest that many patients with alcoholic cirrhosis have folic acid deficiency. One subject with megaloblastic anemia, abnormal FiGlu excretion after 20 gm. histidine, and low serum folic acid level was placed on a diet containing approximately 50 μg. per day of "free" folic acid activity for L. casei. He gradually improved hematologically on this diet and urinary excretion of FiGlu was no longer abnormal. The ingestion of 2 quarts of muscatel per day for four days did not retard this improvement. Serum folic acid activity remained low. This may suggest that (1) folic acid activity does not "build up" in the serum until the folic acid requirements of the tissues are satisfied; (2) measurement of serum folic acid activity may be a more sensitive indicator of folic acid deficiency than measurement of the metabolic products of such deficiency (such as FiGlu).

Blood Lipids in Acute Starvation in Humans. *John F. Mueller and Jack M. Iacono.* Department of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio.

The pattern of lipid changes in red blood cells and serum induced by starvation can be used to study physiologic mechanisms of fat transport, as well as to extend former observations in starving man by newer technics. Four volunteer subjects

abstained from food for ninety-six hours but were allowed distilled water *ad libitum* while maintaining their usual activities. Serum and red cell fatty acids were measured by gas chromatography and alkaline isomerization before, during and after the experimental four-day period. In addition, serum and red cell total fatty acids, total and free cholesterol, lipid phosphorus, neutral fatty acids and serum free fatty acids, total ketones, calcium and glucose were measured at like intervals.

The changes in the major lipid components compared to those of others reported in the literature. The most interesting and previously unreported observations were in the fatty acid patterns. As starvation progressed, a uniform fall in serum dienes occurred accompanied by a rise in all other polyunsaturated fatty acids. Also associated with the drop in linoleic acid is a rise in oleate and to a lesser extent in palmitate and palmitoleate. In red blood cells the fall in linoleate is not observed, but the increase in arachidonate is present.

The interpretation of these changes at this moment is unsettled. The fall in serum linoleate and rise in more saturated acids supports the role of adipose tissue as a ready source of energy, since these are the directions of change one would expect if fatty acids were being mobilized from such depots. The rise in serum of the more unsaturated acids might reflect the body's adaptation response to starvation whereby dienes are converted to "more essential" tetraenes. The changes in red blood cells are known to lag behind those in serum and may well be explained on such a basis.

Metabolism of C^{14} Palmitic and Linoleic Acids in Normal Human Subjects. *Laurance W. Kinsell, Geoffrey Walker and George D. Michaels.* Institute for Metabolic Research, Alameda County Hospital, Oakland, California.

As part of a program designed to evaluate the mechanism of the hypolipidemic effect of linoleic and other polyunsaturated fatty acids, tracer doses of C^{14}-labeled palmitic and linoleic acids, respectively, have been administered intravenously. The distribution of the isotopically-labeled lipids in plasma cholesterol esters, glycerides, phospholipids, and free fatty acids have been determined at frequent intervals during the first eight hours and at daily intervals for one week. The rate of oxidation has also been determined by the $C^{14}O_2$ content of expired air.

Essentially the same metabolic patterns are observed whether the fatty acids are administered in a cottonseed oil emulsion or in albumin solution.

Palmitic acid disappears very rapidly from the free fatty acid fraction compared to a much more gradual disappearance of linoleic acid. Oxidation of both acids is similar initially, but there is subsequent conservation of linoleic acid. The pattern of appearance and disappearance of label from glycerides is much the same in the case of both acids. Minimal amounts of palmitic acid are present in the cholesterol esters in the majority of normal subjects. Significant amounts of linoleic acid are present in the cholesterol esters at twenty-four hours and for several days thereafter. Very appreciable quantities of linoleic acid are present in the phospholipids within five minutes after the intravenous administration of the material, with progressive decrease in concentration over the first eight hours. Significant amounts are present in some normal subjects one week after administration. Small but gradually increasing amounts of palmitic acid are found in the phospholipids during the first eight hours. In most normal subjects none is present after the third day.

From the foregoing, it is obvious that very significant differences exist in the metabolism of a saturated fatty acid (palmitic acid) and a polyunsaturated fatty acid (linoleic acid). The possible relationship of these findings to the hypolipidemic effect of linoleic acid will be discussed as time will permit.

Studies of the Mechanism and Specificity of the Hydrocholesteremic Action of Low Protein Diets in Man. *Robert E. Olson, Seiji Ito, K. Tripathy and Juanita Eagles.* Department of Biochemistry and Nutrition, Graduate School, of Public Health, University of Pittsburgh Pittsburgh, Pennsylvania.

It has been demonstrated in this laboratory that diets containing 25 gm. of protein derived mainly from vegetable sources will lower serum cholesterol in man. (*Am. J. Clin. Nutrition*, 6: 310, 1958). During the past two years the effect of these low protein diets upon serum lipids and lipoproteins has been studied in twelve additional subjects over longer periods of time, and the quantitative nature of the response explored by feeding protein over the range of 0 to 100 gm. per day. It has been found that the effect is not usually noted until the dietary protein is reduced below 35 gm. per day. The mean change in serum cholesterol in this group

receiving 36 per cent of calories from saturated fat was −54 ±9 mg. per cent and the mean change in the β-lipoproteins determined optically in the ultracentrifuge was −130 ±23 mg. per cent. The adding of choline, tryptophan, and lysine to the basal diet containing 25 gm. protein in amounts equivalent to the control diet did not change the nitrogen balance or the depressed level of serum cholesterol. The level of dietary cholesterol in the range of 0.1 to 1 gm. per day likewise appeared to be unimportant.

A study of the mechanism of the hypocholesterolemic effect of the low protein diet using acetate-1-C^{14} as a label for serum cholesterol was carried out in one patient on diets containing 100 and 25 gm. of protein. It was found that the exit of cholesterol molecules from the plasma compartment exceeded their entry during the low protein period even though both rates were increased. This conclusion was supported by the finding of an increased excretion of radioactivity in the bile acid and sterol functions of the stool during the low protein period.

The Influence of Dietary Fat, Nicotinic Acid and Neomycin on the Fatty Acid Composition of Serum Cholesterol Esters. *James G. Hamilton, James E. Muldrey, O. Neal Miller and Grace A. Goldsmith.* Tulane University School of Medicine, New Orleans, Louisiana.

Patients with moderate hypercholesterolemia were studied on a metabolic ward and received one of two isocaloric diets containing 25 gm. of fat in natural foods and either 90 gm. of saturated fat or 90 gm. of unsaturated fat. Nicotinic acid (1 gm. three times a day) and/or neomycin (0.5 gm. four times a day) were administered during one or more experimental periods (three weeks) of each dietary regimen. Caloric intake was sufficient to maintain body weight constant. Fasting blood samples were drawn twice weekly, the serum separated and stored at −20 degrees. Cholesterol esters were assayed by quantitative glass paper chromatography in the following groups: palmitate, oleate, linoleate, and arachidonate.

A diet high in linoleate caused no marked change in cholesterol linoleate concentration despite a concomitant drop in free and esterified cholesterol; however, there was a reduction in cholesterol oleate, palmitate, and arachidonate. Nicotinic acid therapy during the saturated fat regimen lowered both free and esterified cholesterol but had only minor effects on fatty acid composition of cholesterol esters. When neomycin and nicotinic acid therapy were combined (patient receiving saturated fat), cholesterol linoleate concentrations decreased although cholesterol palmitate, oleate, and arachidonate were not affected. A severalfold increase in fecal excretion of bile acid occurred when neomycin was administered, and substitution of unsaturated for saturated fat during combined nicotinic acid and neomycin administration resulted in dramatic depression of the levels of cholesterol palmitate, oleate, and arachidonate. These data suggest that accelerated turnover of cholesterol, as evidenced by large increases in fecal excretion of steroids, may have a selective effect on the turnover of individual cholesterol esters.

Some Metabolic Effects of Tetracycline. *Maurice E. Shils.* Sloan-Kettering Institute for Cancer Research, Memorial and Ewing Hospitals and Sloan-Kettering Division of Cornell University Medical College, New York, New York.

Tetracycline drugs have been noted by various investigators to cause an increase in blood nitrogen levels in certain patients and experimental animals. The most detailed study on man has been performed by Gabuzda et al. (*Arch. Int. Med.*, 101: 476, 1958) who noted marked increases in urinary nitrogen and riboflavin excretion in malnourished males who were given various tetracycline antibiotics while undergoing metabolic studies. These findings were ascribed to a "catabolic" action of the drugs. Interest in the metabolic basis and clinical implications of these observations led us to further investigation. Metabolic balance studies and serum electrolyte and pH measurements have been carried out on hospitalized patients. Observations were made before, during and after ingestion or injection of amounts of tetracycline within accepted therapeutic ranges while the patients were on rigidly controlled dietary intake. To date five subjects have been studied in detail. In response to the administration of tetracycline for six- to fifteen-day periods, there was a decrease in existing positive nitrogen balance or development of definite negative nitrogen balance in confirmation of previous studies. Of special interest were the following additional observations: (1) Potassium balance changes usually did not follow the negative course expected to occur with the increased excretion of nitrogen. (2) In three of the five patients significant negative sodium balances developed as a result of increased urinary excretion of this ion. (3) The two patients with pre-existing elevated blood urea nitrogen levels had a twofold rise in blood urea nitrogen and

evidence of increasing acidosis associated with the administration of tetracycline.

On the basis of the discrepancy between nitrogen and potassium balance noted in this study, and published evidence of the role of tetracycline in the bacterial cell, it is suggested that this group of drugs exerts an "anti-anabolic" rather than a "catabolic" action in metabolism. The cause for the sodium losses noted is not known but may reflect changes in renal tubular function.

Carbohydrate, Fat, and Nitrogen in the Stools of Control Subjects and Patients with Malabsorption. *Roberta E. Bleiler, James A. Clifton and Harold P. Schedl.* Departments of Nutrition and Medicine, State University of Iowa, Iowa City, Iowa.

Adequate data are available in the literature on fecal excretion of fat and nitrogen by patients with malabsorptive syndromes. However, there is little information on the fecal excretion of carbohydrate by these patients. We have determined the fecal excretion of carbohydrate, fat, and nitrogen in a group of eleven control subjects and nine hospitalized patients with various malabsorptive diseases. Metabolic balance studies were completed on seven control subjects and six patients. Dietary intake was not controlled in the other studies but all subjects were on general diets with the same calorie range as those undergoing balance studies, e.g., 3,000 to 4,000 calories per day. Nitrogen was determined by the macro Kjeldahl procedure, fat as ether-soluble material extractable in a Goldfisch apparatus, and total calories by burning aliquots of dried stool in an oxycalorimeter. Protein and fat calories were calculated from the stool nitrogen and fat analyses. Carbohydrate calories were calculated as the difference between total stool calories and stool protein and fat calories. Protein, fat, carbohydrate, and total stool calories were then expressed as per cent of food calories.

In the control subjects, total stool calories ranged from 87 to 258 and did not exceed 8 per cent of the caloric intake. In patients with malabsorption syndromes on a general diet, stool calories ranged up to 25 per cent of the intake. In a patient with Whipple's disease receiving a formula based on milk solids, total stool calories were 45 per cent of the ingested calories. In a patient with post-gastrectomy steatorrhea stool fat calories decreased from 215 to 37 when given a pancreatic extract, but carbohydrate calories changed only from 253 to 175. Two patients with Whipple's disease were given formulas containing skim milk solids and the type of fat was varied. The changes in stool carbohydrate calories did not parallel the changes in fat calories.

Patients with malabsorptive syndromes lose excessive amounts of carbohydrate in the stool, and this loss may vary independently of fat loss.

Effect of a Prudent Reducing Diet on the Serum Cholesterol of Overweight Middle-Aged Men. *Norman Jolliffe, Seymour H. Rinzler, Morton Archer, Ethel Masiansky, Florence Rudensey, Martha Simon and Alice Faulkner.* The Diet and Coronary Heart Disease Study Project, Bureau of Nutrition, New York City Department of Health, New York, New York.

In our fifty to fifty-nine year age group, 207 obese men were placed on a prudent type reducing diet: calories averaging 1,600, protein 140 gm., carbohydrate 180 gm., and fat 33.5 gm. (19 per cent of total calories) with saturated, monounsaturated and polyunsaturated fatty acids supplying 7.8, 6.5, and 4.7 per cent of total calories, respectively. The addition to this diet of 45 gm. of corn oil daily plus more fruits and vegetables if required converts it to the prudent diet previously described.

Of this starting group, 111 adhered so rigidly to the prescribed diet as to achieve a Performance Index of 85 or better during the first ten weeks of observation. Their average control serum cholesterol dropped from 261 to 225 mg. per 100 ml. by the end of six weeks and remained level thereafter. Of the 111 men, ninety-seven reduced to desirable levels, seventy-four of these have been maintained on the prudent maintenance diet for at least six months, and fifty-six of these maintained for one year with the average serum cholesterol remaining level.

The average cholesterol level of the top, middle and low thirds fell from 307, 251 and 207 mg. per 100 ml., respectively, to 254, 222 and 189 mg. per 100 ml., respectively. Maintenance of weight (after successful weight reduction) by use of the prudent maintenance diet serves to maintain the lowered cholesterol level and prevents its rebound. The lower limit reached by each tertile corresponds very closely to its counterpart in the men of normal weight with the major point of difference being the earlier fall achieved on the reducing diet.

Effect of Diet and of Intestinal Microorganisms on Fecal Fatty Acids in Man. *Barbara Underwood, Sami A. Hashim and Theodore B. Van Itallie.* Department of Medicine, St. Luke's

Hospital and Institute of Nutrition Sciences, Columbia University, New York, New York.

Fatty acid composition of fecal lipids was studied in normal subjects consuming formula-type diets both with and without a source of dietary fat. Antibiotics were given to sterilize the bowel in an attempt to study effect of flora on quantity and character of excreted fecal fatty acids. Lipids were extracted from fecal specimens and analyzed for total fatty acids, and by gas-liquid chromatography for identification of specific fatty acids. Subjects were studied while consuming *ad libitum* diet, formula diet containing corn oil as the exclusive source of fat, fat-free formula, and fat-free formula with supplemental antibiotics.

In subjects consuming fat-free diets, there was a significant decrease in the amount of daily fecal fatty acids (0.5 gm.) as compared with those consuming corn oil formula diets (3.5 gm.). Predominant fecal fatty acids, regardless of regimen, were $C^{16:0}$, $C^{18:0}$, $C^{18:1}$, and $C^{18:2}$. Pattern of fatty acids excreted was not directly related to that of ingested fatty acids. Absence of fat in the diet was associated with increased excretion of $C^{16:0}$, and $C^{18:0}$ fatty acids. This pattern of fatty acid excretion was not altered by the administration of antibiotics. It is concluded that on a fat-free diet, the pattern of fecal fatty acids is not significantly affected by elimination of intestinal microorganisms by antibiotic therapy.

On the Protein Allowances for Premature Infants. *B. M. Kagan, Natalia Felix, Charles Molander and Julianne Hill.* Departents of Pediatrics, Cedars of Lebanon Hospital, and University of California at Los Angeles School of Medicine, Los Angeles, California.

The lack of agreement on recommended protein allowances for premature infants is reflected in the absence of a firm recommendation in the last revision of recommendations published by the Food and Nutrition Council of the National Research Council in 1958. Some of the strongest support for a high protein allowance comes from the fact that when fed high protein-containing cow's milk mixtures, premature infants gain more weight than those fed the lower protein intakes provided by human milk.

Data have been collected which show that once prematurely born infants attain a weight of 2,500 gm. varying the protein intake from concentrations provided by human milk to more than double this amount results in no differences in weight gain. The most impressive differences in weight gain with changes in protein intake are found in the smaller and younger premature infants, especially those under one month of age and those weighing less than 1,600 gm. at birth. Differences in weight gain among such infants may be more closely related to the ash intake than to protein intake. The hypothesis has been proposed that this in turn represents water retention based upon renal insufficiency. This is further suggested by evidence showing that renal maturation is related to the size of the infant and to gestational development and also by the fact that premature infants having reached a maturity represented by a weight of 2,500 gm. no longer show such differences in weight gain with changes in mineral intake.

Measurements of body water changes in small premature infants fed human milk or high protein, high ash, low fat cow's milk in isocaloric amounts were made. The data show that the infants fed human milk show changes which correspond with the direction of normal growth, i.e., loss of percentage total body water and extracellular water. Those fed the cow's milk gained in percentage of water in both compartments. The gain in per cent extracellular water among those fed the cow's milk is significantly different and accounts for approximately one third of the difference in weight gain.

Increased weight gain with high protein intake is obviously not enough to support the view that high protein allowance is desirable. The character of the weight gain and other possible correlates must be considered in any analysis of its significance in determining recommended nutritional allowances.

Lysine Supplementation of White Flour. *Elmer L. Severinghaus and W. Henry Sebrell, Jr.* The Institute of Nutrition Sciences, Columbia University, New York, New York.

Feeding 150 gm. daily of white enriched bread with 625 mg. lysine hydrochloride per 100 gm. of flour to 201 Haitian children for 150 days through the school year of eight months was followed by a gain in stature and in weight. The rate of gain in stature was greater during the first four months than subsequently. Comparisons were made with another school where 135 children received the same amount of bread, identical save for the omission of lysine; and with a third school where 128 children received no supplemental food. Differences in weight increments and in the skinfold thickness were observed.

These data suggest that supplemental feeding of undernourished children between ages five and thirteen years may lead to significant acceleration

of growth within four months, and that weight increments vary with the amino acid adequacy of the diet as supplemented. The lysine-enriched bread feeding was associated with a demonstrably greater conservation of skinfold fat in growing children than that following feeding the standard bread.

The incidence of malaria in these children was low. Intestinal parasitism does not appear correlated with hemoglobin levels, but may be a significant factor in the substandard heights and weights for age.

Effects of Chronic Excess Salt Feeding. Enhanced Hypertensogenic Effect of Sea Salt Over Sodium Chloride. *Lewis K. Dahl and Martha Heine.* Medical Research Center, Brookhaven National Laboratory, Upton, New York.

Feeding of excessive sodium chloride has been shown to produce experimental hypertension in the chick, rabbit and rat. Although the role of dietary salt is less firmly established in the etiology of human hypertension, considerable evidence has been adduced in our laboratory to support this thesis.

All these studies have tended to emphasize the importance of sodium ion to the exclusion of other factors. However, salt, as actually used by various peoples, often is quite impure. This raises the possibility that the hypertensogenic effect of sodium in human populations might be modified by additional elements. For example, a study in 1958 showed that hypertension is common among the Japanese, where average salt consumption is high, ranging from about 14 gm. in the south to about 27 gm. in the north. Their salt, derived by processing sea water, usually contains a mixture of substances.

In the present study female Sprague-Dawley rats were fed two kinds of high salt diets for one year. One group of twenty-eight animals was fed a diet containing 8 per cent chemically pure sodium chloride; the other group of thirty-six members was given approximately the same amount of sodium chloride in 11.6 per cent sea salt. Blood pressures and weights were measured at monthly intervals. Food consumption was measured periodically. No differences in either body weights or food intake between the two salt-fed groups were observed. Histologic examination of animals sacrificed during the course of the study is still in progress but thus far significant differences between the two groups have not been noted.

After one year on these regimens the colony of female Sprague-Dawley rats on diets containing sea salt had about four times as many members with blood pressures in excess of 180 mm. Hg as did the colony consuming diets containing sodium chloride alone. The factors involved in this incremental effect have not as yet been identified. This work was supported by the U. S. Atomic Energy Commission.

Careers for Internists in Nutrition Research. *Donald M. Watkin.* Pan American Health Organization, World Health Organization, Mexico, D.F., Mexico.

Few specialties within the field of internal medicine offer the trained physician the challenge and the opportunity afforded by research in the field of nutrition. This is due in large part to the current neglect by adequately trained physicians of this discipline in medical science. This neglect has led in turn to the assumption of leadership in nutrition research by many who are not physicians or by physicians who have no specialized training in internal medicine. The desirability of highly trained physicians assuming leadership is apparent from the ultimate objective of all nutrition research, *viz.*, the elucidation of optimum nutrient intakes for all ages, all normal physiologic states and all disease entities of man. The beneficiaries of such research may be found in all nations and all climates and among all races, economic classes, ages, physiologic states and degrees of health. The advances in knowledge made during studies of nutrition in technically underdeveloped countries may well provide the code for deciphering the riddles posed by the chronic metabolic illnesses prevalent in more technically advanced nations. It is essential, therefore, that future nutrition investigators be well grounded in basic science and clinical medicine so that opportunities for such cross fertilization may not be lost. No specialist is more suited than the internist to assume leadership in this field.

Today, in only one portion of the Americas, the lands surrounded by and bordering the Caribbean, there are at least half a dozen opportunities for internists interested in nutrition research to develop clinical, laboratory and field programs and, in the process, to achieve world-wide reputations. Similar opportunities exist wherever actual undernutrition is a problem. In addition, unlimited opportunities in nutrition research exist in the field of chronic illness, in such diseases as atherosclerosis, cancer, diabetes and genetically determined metabolic disorders. Opportunities for initiating modern investigations of the interrelations of nutrition and the aging process are innumerable. For the internist with a bent for technical development, food technology and its integration into well rounded nutrition research programs afford a challenge.

Today the greatest need in the field of nutrition is not for money, buildings, equipment or clinical material but for well trained, hardworking, dedicated physicians who can apply their specialized training in the laboratory and clinic and their energies and personalities to seeking the optimum nutrient intake for all segments of mankind. Qualified physicians wanting challenge, opportunity and satisfaction from their careers need have no fear of adequate support in nutrition research. The opportunities will seek out all who display genuine interest.

A New Method for the Bioassay of Appetite Suppressants. *G. Friedman* (by invitation), *L. A. Weingarten* (by invitation) *and H. D. Janowitz.* Division of Gastroenterology, Department of Medicine, The Mount Sinai Hospital, New York, New York.

At present appetite-suppressing drugs designed for obese patients are screened in normal animals. In addition, available animal assay methods lack the uniformity necessary for adequate qualification. The present study was designed to overcome these limitations by using hyperphagic mice made obese by aurothioglucose (regulatory obesity). The method provides (1) a simple unique means of screening compounds with regard to appetite suppression in obese and nonobese animals, (2) an index for correlating central nervous system responses (locomotor activity) with appetite suppression, (3) a method for evaluating "tachyphylaxis" of sympathomimetic amines, and (4) a means of testing cumulative or delayed effects of appetite suppressants.

Aurothioglucose obese mice and litter-mate nonobese mice were trained to consume their daily food intake in an eight-hour period. Obese animals ate 30 per cent more food per day than control animals. Following an adequate control period, daily graded doses (based on the estimated lean body mass) of various appetite suppressants were administered for four- to five-day periods and food intake compared with the corresponding control period. One week rest intervals allowed the animals to return to their previous levels of food intake and weight. Activity patterns (locomotor activity) were determined five to ten minutes following the subcutaneous administration of medication.

Dextroamphetamine (5 mg. per kg.) was employed as the standard, and depressed food intake 23 per cent in control animals and 30 per cent in aurothioglucose obese mice, with 4-plus locomotor activity patterns. Dl-amphetamine produced similar suppression of food intake and activity patterns at a dose of 10 mg. per kg., while phenmetrazine produced equivalent results at 15 mg. per kg. Diethylpropion was ineffective in these dose ranges. In general, appetite suppressant effects were paralleled by increased locomotor activity. The relative doses employed correlate well with results of double blind studies in human obese subjects. This study was aided by grant AT-501 from the National Institutes of Health.

Effect of Vitamin B_{12} on Hemolysis in Pernicious Anemia. *Edward H. Reisner, Jr.* Department of Medicine, St. Luke's Hospital, New York, New York.

Pernicious anemia is a hemolytic anemia caused by deficiency of vitamin B_{12} leading to abnormal megaloblastic blood formation. It has been assumed that the observed shorter survival time of pernicious anemia macrocytes accounted for the hemolytic factor in this disease. That there might be a plasma hemolytic factor, as well, has been suggested by several authors.

Three patients with pernicious anemia were observed in whom sustained reticulocytosis without accompanying rise in the blood count was clear evidence of dyspoiesis with new blood cells being destroyed as rapidly as they were formed. When vitamin B_{12} was administered there was prompt restoration of effective hematopoiesis with accompanying fall in bilirubin and urobilinogen levels and rapid rise of hemoglobin and red blood cell count.

Survival of Cr^{51}-labeled erythrocytes was studied in seven patients with pernicious anemia in relapse. In six patients whose own cells were labeled and reinjected prior to, or immediately following, specific therapy observed half-lives were eleven, eleven, twelve, thirteen, twenty-three and twenty-nine days. By taking frequent determinations it was possible to demonstrate a double sloped curve in four subjects, with the most rapid cell disappearance occurring prior to or up to four days after vitamin B_{12} therapy. Values obtained for the respective half-lives were $^{7}/_{15}$, $^{5}/_{14}$, $^{7}/_{14}$ and $^{9}/_{25}$ days. In a seventh subject whose own cells had a half-life of sixteen days in a normal recipient, transfused normal cells had a half-life of nineteen days before and thirty days after treatment was instituted. The cells of one of the other patients with a half-life of thirteen days survived twelve days in a normal subject. In the normal recipient the pernicious anemia cells showed a single slope rate of disappearance.

These data are interpreted as confirmatory evidence of a vitamin B_{12} deficiency-induced hemolytic

factor in the plasma of many patients with pernicious anemia. Possible mechanisms will be discussed.

Clinical Variations in Vitamin B_{12}-Deficiency Anemias. *S. O. Waife, O. M. Helmer and P. J. Fouts.* Lilly Laboratory for Clinical Research, and the Marion County General Hospital, Indianapolis, Indiana.

In recent years, four patients have been seen in the hematology clinic at Marion County General Hospital whose response to the Schilling test suggests unusual clinical variations in vitamin B_{12}-deficiency anemia. The laboratory findings closely resemble classic pernicious anemia, but certain features suggest a new classification may be needed.

All four patients had a macrocytic hyperchromic anemia, megaloblastic bone marrow, and clinical features typical of pernicious anemia. They showed a prompt reticulocyte response to therapy (liver, vitamin B_{12} or vitamin B_{12}-intrinsic factor concentrate) and were adequately maintained for years.

One patient represented a classic uncomplicated case. After years of therapy, a Schilling test was performed which indicated normal absorption of vitamin B_{12}. Another patient had a relative deficiency of intrinsic factor, in that the Schilling test showed some absorption which could be augmented by exogenous intrinsic factor. Both had histamine-fast achlorhydria (pH over 7). No other cause for the anemia was discovered. Was this a vitamin B_{12}-deficiency anemia in the presence of intrinsic factor?

Two patients had no vitamin B_{12} absorption (Schilling) even on large doses of hog intrinsic factor. Both failed to respond to normal human gastric juice, but while one had true achlorhydria, the other had a gastric pH of 1.2. However, gastric juice from the patient with gastric acidity was active in another patient with pernicious anemia. In none of these was there evidence of significant malnutrition or true malabsorption.

These observations, to be reported in detail, indicate that there are patients with findings clinically similar to typical pernicious anemia, who might have a positive reaction to the Schilling test or who might have a negative reaction to the Schilling test after normal gastric juice. These patients represent probable instances of vitamin B_{12}-deficiency anemia which do not fit into the usual categories of pernicious anemia (intrinsic factor deficiency), nutritional macrocytic anemia, or malabsorption syndromes.

Reviews of Recent Books

Nutritional Evaluation of Food Processing, edited by Robert S. Harris and Harry von Loesecke. John Wiley & Sons, New York, 1960, pp. 612, $12.00.

The publication "explosion" in scientific literature drives the specialist into ever narrowing areas. The production of a comprehensive review is best left to a number of competent authorities rather than one or two. Here we have thirty-three authors, each a specialist in his assigned field. The editors have capably merged the material into an organized whole, with relatively little overlapping. The text is clear, concise and easily followed, in part due to the use of frequent subheads in bold type. To each chapter is appended a bibliography, listing as many as 300 references.

As the title suggests, the book is devoted to the processing of foods and the nutritional implications. In addition, there is a fifty-page review on how agricultural practices affect food composition. Also, the discussions of food values go well into some of the basic concepts of nutrition as revealed in recent research, e.g., amino acid essentiality and imbalances, antimetabolites, trace minerals, etc. The coverage is up-to-the-minute; ionizing radiation sterilization is given eleven pages.

A good index is extremely important in a text to be used frequently for reference. We can rate this one perhaps better than average. There are a number of obvious omissions. For instance, the page which carries the most information about margarine is not listed at all. The effect of radiation sterilization on food utilization is well discussed on page 537, a reference not to be found in the index. In several cases, all inclusive subjects should be broken down into subtopics. Of great assistance to the searcher after specific information, however, should be the detailed table of contents. There, under the thirteen chapter headings are listed some 200 subheads.

In some instances the authors fall into an error often noted in other publications where before-and-after-processing data are compared. The use of laboratory or pilot plant facilities in transforming raw foods into storable products can produce more drastic changes then occur in commercial practice. The laboratory-produced canned milk, for instance, is not the same as that purchased on the open market. A statement on page 411 in another connection could well apply here: "Conclusions based upon such studies should not be extended beyond the scope of conditions existing in the study."

There are a few disappointing omissions. Hydrogenation of fats is discussed in detail from the chemical point of view (six pages); the nutritional implications are scarcely mentioned.

The text does well what it set out to do, document the handling and processing of food "from garden to gullet" in its scientific aspects. The book is of irreplaceable value to the researcher, teacher, student and writer. It is hoped that those writers who love to portray to the public the "destruction" of food values in processing will read and heed.

FRANK E. RICE

Medicinal Chemistry, 2nd edition, edited by Alfred Burger. Interscience Publishers, Inc., New York, pp. 1,243, $37.50.

The practice of medicinal chemistry as an art or science has grown enormously in the past decade. Although the volume of published data is staggering, much important and relevant material, particularly crucial negative findings, remains buried in the files of industrial firms and in the minds of individual chemists. Any attempt to make a coherent and comprehensive statement of the current status can at best achieve only partial success. This long, heavy and expensive text serves to confirm this judgment.

Dr. Burger has called upon thirty-five associates to prepare chapters on special subjects since one man is no longer capable of preparing a book of this sort. The various chapters are of unequal length and completeness. Some are merely brief summaries of some outstanding developments, while others appear to be relatively thorough surveys. The section on vitamins represents a good summary of the chemical knowledge in this field. In contrast, other chapters such as those on diuretics, hypnotics and sedatives, merely skim the surface.

It is regrettable that a text published in 1960 covers the literature only up to the middle of 1957. One might reasonably have expected a better quality of printing in a text of this price. Another more serious criticism is perhaps inherent in the nature of the material covered. Since it is manifestly impossible for a book of this sort to be really complete in coverage, the alternative is to utilize selected examples to illustrate the principles of the practice of the science. Such a text, while lacking the detailed coverage of a reference work, would be more useful for it could present the reader with a better understanding of the philosophy and technics of the medicinal chemist.

Regardless of its limitations, the book is relatively unique in its coverage and will continue to serve as a starting point for many wishing to survey the literature in selected areas. I. H. SLATER

Clinical Endocrinology, I, edited by E. B. Astwood. Grune & Stratton, New York, 1960, pp. 724, $18.75.

This valuable book of sixty-eight chapters is an expansion and revision of a volume, *Progress in Clinical Endocrinology,* published ten years ago. The authors are eighty-two experienced investigations and each treats his section with an individualistic approach which in many ways is refreshingly novel. For example, the seven chapters on the pancreas include mechanism of action of insulin, glucagon, fat metabolism in experimental diabetes, nonpancreatic diabetes, oral hypoglycemic agents (two) and hypoglycemia associated with tumors. In other words certain areas in the general field of endocrinology were selected for special discussion. These subjects would seem to be those in which significant progress has been made recently.

Both experimental and clinical chapters are presented. There are 169 illustrations and a detailed index.

This is not a standard textbook of endocrinology and thus the title may be somewhat misleading. A number of endocrine disorders are not mentioned. Nor, for example, will the reader find a discussion of the insulin treatment of diabetes. On the other hand, as a collection of diverse essays on certain aspects of endocrinology, this book is up to date, wide in range and excellent in quality. S. O. W.

Basic Facts of Body Water and Ions, by Stewart M. Brooks. Springer Publishing Company, New York, 1960, pp. 159, $2.75.

This small, soft cover book is intended for the student and practitioner of nursing, medical technology and pharmacy. It therefore is written in a simple direct style and presupposes little background information on the part of the reader. It attempts to review the basic facts in physiology and biochemistry, and a little clinical medicine, relating to fluid and electrolyte balance, edema, renal failure, etc.

While much information is straightforward, there are several weak spots which one should recognize. Thus the "low salt" syndrome is said to be the most common fluid-electrolyte derangement, nothing is said about parenteral fat solutions, and certain pharmaceutical preparations are singled out for mention to the exclusion of others. "Calcium should be avoided whenever possible" in a digitalized patient, etc.

In view of the many monographs on this complex subject, one must perforce compare this book to its "competitors." In the reviewer's opinion, there are several better books available on the subject.

S. O. W.

Annual Review of Medicine, Vol. 11, edited by David A. Rytand. Annual Reviews, Inc., Palo Alto, 1960, pp. 453, $7.00.

The 1960 edition of this well known series contains a chapter on nutrition by Jolliffe and Goodhart. Special attention is given to the lipid-atherosclerosis problem and to recent contributions to our knowledge of vitamins. As usual, these volumes are a most useful compendium of significant papers, and hence invaluable for a quick survey of the many active fields of medicine.

S. O. W.

Medical Surveys and Clinical Trials. Some Methods and Applications of Group Research in Medicine, edited by L. J. Witts. Oxford University Press, New York, 1959, pp. 325, $8.00.

Despite the vast expansion in the field of *clinical* research there are surprisingly few books on the topic. This multi-authored volume concentrates on "group research" as distinguished from the intensive study of a few individuals. Both approaches, of course, are absolutely essential for the further progress of medicine.

In an earlier day, workers in the field of public health and tropical medicine had already explored this fertile area. Soon nutrition scientists (e.g., in pellagra and beriberi) were developing studies on large population groups. With the advent of sulfonamides and antibiotics the whole field of therapeutics took on a new look. It now became necessary to support one's conclusion with "figures."

Professor Witts of the University of Oxford has gathered a group of able and experienced scientists and together they have produced a highly informative well written and important book. Among the subjects covered are retrospective and prospective studies, placebos, prophylactic and therapeutic trials and prevalence surveys. Methods are discussed in the first half; their application to various clinical states in the second. Our readers will be especially interested in Sinclair's chapter on "The Assessment of Nutriture" and the comments by Bronte-Stewart and Pickering on cardiovascular diseases. Equally interesting are chapters on child development, genetics, cancer and mental diseases among others.

Those interested in human nutrition can well appreciate the value of research on population groups. Much of what is current in our thinking about lipids and atherosclerosis, infant feeding and kwashiorkor, for example, are based on dietary surveys and wide-range therapeutic trials. This book, therefore, should be read by all who undertake work involving numbers of patients. It is a valuable addition to a vital segment of clinical research.

S. O. W.

Abstracts of Current Literature

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PHYSIOLOGY OF GASTROINTESTINAL FUNCTION

The regulation of appetite and of gastric hunger contractions has been a controversial topic. Is the important factor the volume of food, its caloric value, its carbohydrate or protein content, or change in body fluids caused by absorption of digestive products? According to Mayer, the availability of glucose as a source of energy with certain sypothalamic centers of the central nervous system reprehents the primary controlling factor in this relationship.

Relation of Metabolic Events to Gastric Contractions in the Rat. S. Sudsaneh and J. Mayer. *Am. J. Physiol.*, 197: 269, 1959.

A water-filled balloon system was used to study gastric hunger contractions in normal rats. Fasted rats have active hunger contractions alternating with periods of quiescence. The contractions occur at a rate of one or two per minute for ten to fifteen minutes followed by a quiescent period of five to ten minutes. Exposure to cold (5°c.) and heat (32° and 40°c.) inhibited gastric hunger contractions in fasted rats. The intravenous administration of 75 μg. glucagon or the intravenous injection of 50 μg. epinephrine and norepinephrine also inhibited gastric hunger contractions. Cessation of the gastric motility also occurred when 100 μg. glucagon or 50 μg. epinephrine were given to fed animals pretreated with 2 units of insulin. It was found that changes in carbohydrate utilization induced by glucagon preceded the gastric phenomena. AUTHORS

Mechanism of Hypothalamic Control of Gastric Contractions in the Rat. J. Mayer and S. Sudsaneh. *Am. J. Physiol.*, 197: 274, 1959.

Gastric hunger contractions in hypothalamic hyperphagic and aphagic rats are similar to those of normal rats. Heat, cold and epinephrine inhibited hunger contractions in both types of hypothalamic animals. The gastric hunger contractions of aphagic rats are inhibited normally by glucagon. However, doses of glucagon which inhibit hunger contractions in normal rats fail to do so in hyperphagic hypothalamic animals whether obese or reduced to normal weight, despite the fact that responses are similar to those seen in normal animals. This ventromedial "glucoreceptive" hypothalamic area thus seems to exert a considerable measure of control upon gastric hunger contractions while the lateral area does not. A scheme integrating hypothalamic, metabolic and gastric factors in hunger is proposed. AUTHORS

Effects of Some Physiologic Substances on Gastrointestinal Propulsion in the Rat. P. M. Lish, B. B. Clark and S. I. Robbins. *Am. J. Physiol.*, 197: 22, 1959.

The influence of some physiologic substances on the distribution of a dye following oral administration in the rat has been studied by a very sensitive and discriminative technic. Under certain conditions an agent may influence the rate of movement through one portion of the gastrointestinal tract while not affecting the transit through an adjacent segment. Propulsive motility was slowed following subcutaneous administration of *l*-epinephrine, *l*-norepinephrine, glucagon, 5-hydroxytryptamine, or 5-hydroxytryptophan. The characteristics of the inhibition produced by each agent were not the same. Insulin inhibited the motility of the gastrointestinal tract fifteen minutes following subcutaneous administration, but forty-five minutes after administration there was intense stimulation of propulsion. Ganglionic blockade eliminated the stimulating effect of insulin on the intestine but only partially reduced the stimulation of the stomach. AUTHORS

The inhibitory effect of a high fat meal upon gastric motility has been recognized for many years. This has been part of the treatment for peptic ulceration using a milk and cream diet which, in intractable cases, has proved superior to protein-supplemented milk formulas.

The Effect of an Ordinary and of an Excessively Fatty Breakfast on Human Gastric Antral Motility. A. W. M. Smith and C. F. Code. *Gastroenterology*, 35: 398, 1958.

The gastric antral motility of twelve healthy human subjects was recorded for three-hour periods by a balloon-photokymographic technic during fasting and after the ingestion of a breakfast containing 16 gm. of fat. Similar studies were made of five of the twelve subjects after ingestion of a breakfast containing 100 gm. of fat. Immediately after the breakfast containing 16 gm. of fat, type II waves were reduced significantly as compared to the fasting records, and rhythmic type I activity increased. Total activity was significantly increased during the second hour. After the breakfast containing 100 gm. of fat, type II waves were abolished within fifteen minutes and remained depressed. Type I activity was immediately increased and became more rhythmic.

The mean amplitude of the pressure produced in the balloon by type II waves was 14.3 cm. of water during fasting, 10.3 cm. after the ordinary meal, and 7.8 cm. after the fatty meal. This indicated a significant decrease in the amplitude of the type II waves after both types of meals.

The present study confirms the previously described transient immediate decrease in parastaltic activity occurring when food enters the stomach followed by a gradual increase in parastaltic activity. It also documents the inhibitory effect, on gastric motility, of a meal high in fat previously demonstrated radiologically.

J. B. HAMMOND

The Effect of Bland Fluids and Anticholinergic Drugs on the pH of Gastric Contents in Duodenal Ulceration. T. J. Thomson. *Clin. Sc.*, 17: 701, 1958.

The use of a bland diet in the treatment of peptic ulcer is traditional and indeed is symptomatically very effective. However, it has been shown that the rate of healing and the relapse rate are unaffected by such a diet. The investigation reported here was designed to assess the effect of frequent bland fluid feeds, atropine sulphate, sodium penthienate and continuous intragastric milk drip in twelve men and three women with active duodenal ulceration.

The pH of the gastric contents was measured hourly during treatment in samples obtained by an indwelling Ryles tube. It proved impossible by the means used to raise the pH above 4 and it is concluded that any benefit due to bland diets is unlikely to be related to their effect on gastric acidity. F. E. HYTTEN

Thiamine-deficiency neuritis affects not only the peripheral nervous system but also the autonomic system. This is evidenced not only by changes in motility but also by a reduction of reflexly induced gastric acid secretion.

Effect of Thiamine Deficiency on Canine Gastric Secretion of Acid. M. K. Lavers, P. A. Stefanik and C. F. Code. *Am. J. Physiol.*, 197: 253, 1959.

A study was undertaken to determine the effect of thiamine deficiency on the hydrochloric acid output of vagally denervated gastric pouches (Heidenhain-type) and vagally innervated gastric pouches (Pavlov-type) in dogs. Responses of both types of pouches to injection of 0.05 mg. of histamine/kg. of body weight and the maximal secretory capacity of both types after histamine therapy were unaltered during the deficiency state. A degree of thiamine deficiency sufficient to produce anorexia and neuritis was without effect on the secretory response of canine gastric mucosa to the administration of histamine. The hydrochloric acid output of vagally innervated pouches during nervous stimulation caused by insulin-induced hypoglycemia was drastically reduced as soon as thiamine deficiency developed, while the response to bethanechol chloride was little, if at all, affected. It is concluded that the vagal secretory mechanism participates in the general neural failure of thiamine deficiency and that this failure most likely is in the neurons of the vagal nuclei.

AUTHORS

Inhibiting Effect of Norepinephrine on Gastric Secretion in Human Subjects. A. J. Leonsins and W. R. Waddell. *J. Appl. Physiol.*, 12: 334, 1958.

The action of norepinephrine in relation to gastric secretion was studied on human subject material. The route of administration was by intravenous infusion. When patients were in the basal state norepinephrine inhibited gastric secretion. Norepinephrine was also inhibitory after stimulation of gastric secretion by peptone broth, histamine and hypoglycemia following insulin. In each case it was observed that the volume of gastric juice was reduced. The acidity and chlorides might or might not be reduced and sometimes were increased even when the volume of secretion was less.

M. J. OPPENHEIMER

Adrenal steroids affect gastric activity by increasing acid and pepsin activity. Amphenone may affect these gastric functions through its depressant effect upon adrenocortical activity. In addition, amphenone is a toxic substance which may influence the function of tissues other than the adrenal directly.

Effect of Amphenone on Gastric Secretory Activity. J. Manrique, R. Paredes, J. Arabehety and S. J. Gray. *Am. J. Physiol.*, 195: 221, 1958.

A single subcutaneous injection of amphenone to pyloric ligated rats produced a significant decrease in gastric secretory volume, free acid and potassium content, with an increase in pH, gastric juice sodium and pepsin concentration. Cortisone, corticosterone, DCA or ACTH administered with amphenone did not alter

the amphenone effect. Aldosterone alone produced a reduction in gastric juice sodium. When amphenone was injected with aldosterone, the amphenone effects, including an increased sodium, were again observed. When amphenone was administered with histamine, the amphenone effects relating to pH and free acid concentration were not observed. Adrenalectomy produced a decrease in gastric secretory volume, acidity and pepsin secretion, and an increase in sodium and pH. In previously adrenalectomized rats the amphenone inhibitory effects on gastric secretion were absent. An increase in adrenal gland weight was observed four hours after the administration of amphenone, but was not seen when cortisone, corticosterone, DCA, aldosterone or histamine were administered with amphenone. ACTH, administered alone or with amphenone, produced an increase in adrenal weight. Amphenone caused a considerable increase in thymus weight, which could be prevented by the simultaneous injection of corticosterone, aldosterone or histamine, but not by DCA or adrenalectomy. ACTH or cortisone, administered alone or in combination with amphenone, decreased thymus weight. AUTHORS

Secretory Function of the Stomach After Denervating Operations. W. R. Waddell. *J. Appl. Physiol.*, 12: 468, 1958.

This study deals with acid secretory function in patients with ulcer during control periods and after several types of surgical procedures. In one group of postoperative patients the parasympathetic nerve supply was interrupted. It was observed that the volume of gastric secretion was only slightly increased in these patients after the injection of histamine. On the other hand, insulin hypoglycemia actually decreased the volume of secretion in these patients. However, chloride concentration was increased after both histamine and insulin therapy. The author expresses the opinion that histamine acts principally on gastric blood vessels. Parasympathetic denervation might, in this case, interrupt reflex arcs mediating vasodilation.

The work of Uvnas and others who observed the interrelationship of nervous and humoral control on gastric secretion, while not mentioned in this work, ought to be considered in relation to this report.

M. J. OPPENHEIMER

Attention is called to the recognition of a new congenital defect in the function of the small bowel involving the failure to hydrolyze lactose, possibly due to the absence of lactase.

Defective Lactose Absorption Causing Malnutrition in Infancy. A. Holzel, V. Schwarz and K. W. Sutcliffe. *Lancet*, 1: 1126, 1959.

Failure of infants to gain weight with apparently adequate breast feeding is most often due to an abnormality of milk composition, usually a low fat content. This paper describes two children, a brother and sister, who failed to gain weight on a large intake of breast milk and who had frequent stools and excessive flatus. They made excellent progress on artificial feeds containing no lactose, and tests showed that they were unable to hydrolyze lactose, possibly due to lack of, or inhibition of, the enzyme lactase. Their intestinal symptoms were presumably due to bacterial fermentation of the sugar. This congenital abnormality has not been previously described. F. E. HYTTEN

Intestinal Absorption of Methionine and Histidine by the Chicken. C. M. Paine, H. J. Newman and M. W. Taylor. *Am. J. Physiol.*, 197: 9, 1959.

Intestinal absorption of methionine and histidine was studied in adult chickens having permanent Thiry-Vella fistulas. The L isomers of both amino acids were absorbed from the fistulas more rapidly than were the D isomers. At a concentration of 10^{-3} M, 2,4-dinitrophenol retarded absorption of L-methionine but not of the D form. The absorption of L-histidine was impaired in the presence of equimolar concentrations of either L- or D-methionine. Racemization of D-methionine during absorption could not be demonstrated. The L isomers of both methionine and histidine appear to be absorbed from the chicken intestine by a common specific transport mechanism, with D-methionine being able to participate in at least one stage of this mechanism.

AUTHORS

ITEMS OF GENERAL INTEREST

Progress of the Columbia University Institute of Nutrition Services. AMA Council on Foods and Nutrition. *J. A. M. A.*, 170: 1928, 1959.

This brief report discusses the organization and program of the Columbia University Institute of Nutrition Sciences, Dr. W. H. Sebrell, Jr., Director. It is clear that establishment of this Institute marks and important step in the promotion of nutrition as both a clinical and experimental science. S. O. WAIFE

The Effect of Radiation Sterilization on the Nutritive Value of Foods. V. On the Amino Acid Composition of Milk and Beef. W. S. Tsien and B. C. Johnson. *J. Nutrition*, 69: 45, 1959.

Evaporated milk and beef round steak were used in these experiments. The foods were exposed to radiation at three dosage levels, the highest being 9.3 million rad. The amino acids most seriously reduced were glutamic acid, aspartic acid, serine, and glycine—fortunately all are nonessential. FRANK E. RICE

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Symposium on Additives and Residues in Human Foods

Introduction

MAN is unique among animals in his desire and ability to control his environment. In a broad sense a part of this activity is considered by the Symposium on Additives and Residues in Human Foods. Man has learned to control four and six legged pests in his home; infectious agents in his air, food and water; rainfall from the skies; smog over his cities; nutrient balance of farm soils; and vitamins in his foods. He contemplates control of radiation, of combustion residues and of human fertility. His domestication of plants and animals, their proper feeding and breeding, and the control of their infestations have allowed a small portion of the people to produce abundant food for us all. Progress in these activities has required an arsenal of chemicals. Each compound is intrinsically neither good nor bad; its use may be either good or bad. The quantity used under each condition is important in the reaction obtained. Strychnine is a deadly poison in the hands of a murderer and a life-saving drug when used by the physician.

Food additives are another of man's devices to control his environment for a better life. Man has used spices, salt and herbs on his foods from his first attempts at civilization. The first U. S. patent in food additives was issued in 1886: "A new and useful mixture of salt and phosphate of lime for table use as a condiment for seasoning food."* The population explosion will soon force all nations to produce food to their maximal capacity. Within your lifetime the amount of available food will be the limiting factor in the population growth of these United States. We must learn to use these compounds properly before that time comes.

Many tons of hundreds of chemicals are used each year by the food industry. Food additives are big business. Most additives, however, are not essential nutrients. Each is added for a specific purpose; it may increase yield, growth, efficiency, shelf life or salability It may decrease spoilage, disease, rancidity or losses by predators. Some are unintentional, as material absorbed from containers. Some are gone before the food reaches the consumer, and some leave residues. The main reason for the use of additives is to provide more food at lower unit cost for the producer and/or consumer.

Much time and effort is needed to resolve major questions in the use of additives. This symposium is held in order that you may better evaluate this complex problem. You are vitally involved as a consumer. As one knowledgeable in nutrition you may be asked to resolve dilemmas such as this one. You should transcend emotional and policital vectors to learn the types, usefulness and limitation of these compounds. You should have some idea of the basic chemistry and pharmacology of the compounds involved. You should seek a proper perspective regarding the effectiveness and rationale of present practices and glean from these discussions a positive view of future needs coupled with a realistic philosophy to guide immediate work. Our purpose is to help guide you, tomorrow's leaders, who will face the problems raised today.

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* PETERSON, A. G. Salt and Salt Shakers. Washington, D. C., 1960. Washington College Press.

Additives and Residues in Foods of Animal Origin

H. R. BIRD, PH.D.*

RECENT expressions of opinion by experts on additives and residues in foods have been diverse, to say the least. An example of the diversity of viewpoints on this subject is exemplified by this statement: "If an additive involves any risk whatever to the consumer, it should be prohibited. What if the additive does increase production? We have overproduction already." One answer to this is that malnutrition is still a major world health problem, even though not in this country.

The following example illustrates another aspect of this argument: the New York wholesale price of ice-packed broilers was 32.9 cents per pound in 1930 and 25.1 cents in 1957.[1] The prices in the intervening years show the effect of inflation pulling in one direction and improved technology pulling in the other. Important contributions to improved technology in broiler production are made by four groups of additives: antibiotics, antioxidants, arsenicals and coccidiostatic drugs. There would be unanimous agreement on discontinuance of all of these if hazards to consumers were demonstrated. But do we want to pay substantially more for fried chicken just because someone may suspect a hazard, without even weighing the evidence for and against the suspicion? Should we not try to determine whether the hazard is large, medium-sized, small or imaginary?

In every decision on additives and residues, concern for the public health must be paramount; but if the risk is so small that it can be appreciated only by the elect, the professional viewers-with-alarm, then at least it should be balanced against the economic advantages of the additive in question. In considering the problem of residues, let us not lose sight of the importance of the protein and vitamins which we expect producers to furnish to us at low cost in meat, milk and eggs.

The number of additives to animal feeds is impressive or disturbing, depending on the point of view. Table 1 lists officially acknowledged additives.[2]

ANTIBIOTICS

For the last ten years antibiotics have been widely used at low levels in feeds to stimulate growth of young chickens, turkeys, and pigs. They are used less extensively in feeds for young ruminants. In the last three or four years they have been fed at low levels to many laying flocks to increase egg production. They are also administered at higher levels in feed or drinking water or by injection in the treatment of various diseases of livestock and poultry. In the earlier experiments the feeding of a low level of antibiotic usually resulted in at least 10 per cent greater weight during early growth and also 10 per cent greater efficiency of feed utilization. In recent experiments, the response to antibiotics has often been less. Comparisons made with turkey poults by McGinnis et al.,[3] indicated that the "old" antibiotics had lost their effectiveness and that antibiotics not previously used in that laboratory were effective. Waibel et al.,[4] and Libby and Schaible[5] found with chicks, and Hanson et al.,[6] with pigs, that the effectiveness of antibiotics had diminished, and they concluded that continued use of antibiotics had "cleaned up" the environment so

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* Professor and Chairman.

Presented at a Symposium on Additives and Residues in Human Foods at the University of Missouri School of Medicine, Columbia, Missouri, April 27, 1960.

TABLE I
Additives to Animal Feeds[2]

Additives	Classification*	No. of Days Withdrawn Before Slaughter
Antibiotics		
Aterramin	O.D.	...
Bacitracin, zinc bacitracin	C.A.	...
Chloramphenicol	C.A.	...
Chlortetracycline	C.A.	...
Erythromycin thiocyanate	O.D.	...
Hygromycin B	N.D.	...
Nystatin†	N.D.	...
Oleandomycin chloroform adduct	N.D.	...
Oxytetracycline	O.D.	...
Penicillin, procaine penicillin	C.A.	...
Streptomycin, dihydrostreptomycin	C.A.	...
Tetracycline	C.A.	...
Arsenicals		
Arsanilic acid and Na salt	O.D.	5
Arsenosobenzene	N.D.	...
3-nitro-4-hydroxyphenylarsonic acid†	O.D.	5
4-nitrophenylarsonic acid	O.D.	5
P-ureidobenzenearsonic acid	N.D.	5
Coccidiostats		
Arsenosobenzene	N.D.	...
Bithionol + methiotriazime	N.D.	3
2,4-diamino-5-(p-chlorophenyl) 6-ethylpyrimidine	N.D.	4
3,5-dinitrobenzamide + 3 nitro-4-hydroxyphenylarsonic acid + acetyl (p-nitrophenyl) sulfanilamide	N.D.	5
Di-*n*-butyltin dilaurate	O.D.	...
Furazolidone‡	O.D.	...
Glycarbylamide	N.D.	...
Nicarbazin	N.D.	4
Nitrofurazone‡	O.D.	...
Nitrophenide	O.D.	...
Sulfaquinoxaline	O.D.	4
Hormone-active compounds		
Dienestrol diacetate	N.D.	1
Diethylstilbestrol	N.D.	2
Iodinated casein	O.D.	...
Enzymes		
Enzymes (except pepsin)	N.D.	...
Pepsin	O.D.	...
Tranquilizers		
Reserpine	N.D.	...
Hydroxazine hydrochloride	N.D.	...
Vitamin		
Menadione sodium bisulfite	O.D.	...
Miscellaneous		
Diethylcarbamazine	N.D.	...
2-acetylamino-5-nitrothiazole	N.D.	7
Nithiazide	N.D.	1
Amino-nitrothiazole	O.D.	7
Cadmium anthranilate	O.D.	30
Cadmium oxide	O.D.	30
2,2-dihydroxy-5,5-dichlorodiphenyl methane	O.D.	...
Dinitrophenylsulfonylethylene diamine	O.D.	...
Dried rumen bacteria	O.D.	...
Dynafac	O.D.	...
Nicotine	O.D.	...
p-aminobenzoic acid + Na and K salts	O.D.	...
Phenothiazine	O.D.	...
Piperazine and its salts	O.D.	...
Sodium fluoride	O.D.	...
Sodium propionate	O.D.	...

* C.A. = certifiable antibiotic; N.D. = new drug; O.D. = old drug.

† May not be fed to hens producing eggs for human consumption.

‡ Also used in bacterial infections.

that untreated control animals were growing as rapidly and efficiently as antibiotic-fed animals. In this situation it would be expected that discontinuance of low level antibiotic feeding would result eventually in a return to the lower growth level prevailing before antibiotics were used, and to a very serious decline in efficiency of production of pork and poultry meat. Discontinuance of the use of therapeutic levels would deprive producers of some of their most effective protection against some animal and poultry diseases. Balanced against the very great advantages of these uses of antibiotics in meat production is the almost total lack of residue problem.

"Low levels" of dietary antibiotic have been arbitrarily defined as less than 50 gm. per ton feed.[2] Such low levels of chlortetracycline and oxytetracycline result in no detectable levels of antibiotic in edible tissues of chickens or

pigs according to methods capable of detecting approximately 0.1 μg. per gm. of tissue.[7-9] Levels of tetracyclines ranging from 200 to 1,000 gm. per ton had to be fed before antibiotic could be detected in liver, and levels of 1,000 to 2,000 gm. per ton before antibiotic could be detected in muscle. After feeding zinc bacitracin at a level of 100 mg. per kg. (approximately 90 gm. per ton) Heth and Bird[9] found no antibiotic in liver or muscle by a method capable of detecting 3 to 4 μg. per gm.

Cover and Ludwig[10] found no streptomycin in the livers of chickens after administration of streptomycin sulfate by aerosol or dust. No penicillin was detected in liver two hours after the intramusclar injection of 10,000 units of procaine penicillin G per pound body weight. Doubling the dose permitted detection of penicillin in liver. Streptomycin was detected in liver after intramuscular injection of 100 mg. per pound body weight. Detectable levels of both antibiotics in liver and other tissues were of short duration.

Raica et al.[11] found no chlortetracycline in eggs after feeding hens 200 gm. per ton of feed. Feeding 500 to 2,000 gm. per ton resulted in detectable levels in eggs but all eggs tested negative during the first week after removal of antibiotic from the diet. Thus antibiotics, which are not present in the edible tissues of eggs of antibiotic-fed animals, would be destroyed by cooking if they were present.

Chlortetracycline and oxytetracycline are used in the chilling water in poultry dressing plants to increase the shelf life of the poultry.[12,13] The level of antibiotic found in any part of the uncooked poultry may not exceed 7 p.p.m.[14] The residue is destroyed by cooking.[15] A derivative of chlortetracycline, designated isochlortetracycline, has been found in cooked poultry meat. It has no antibiotic activity.[16]

ARSENICALS

About the same time that antibiotics came into use in animal feeds, two arsenicals also appeared as feed additives. They are 3-nitro-4-hydroxyphenylarsonic acid and arsanilic acid. When fed without an antibiotic to young growing animals either of these compounds affects growth and efficiency of feed utilization in the same manner as an antibiotic. When an antibiotic and an arsenical are fed together, the beneficial effects are not quantitatively additive, but it has been reported that in the diet of young chickens the combination of both is more effective in improving weight and market grade than is the antibiotic alone.[17]

The arsenicals were first permitted in poultry and swine feeds after it was demonstrated that feeding the effective levels resulted in residues of arsenic in the edible tissues not over 0.5 to 1.8 p.p.m. in chicken and turkey liver, 0.06 to 0.75 p.p.m. in chicken or turkey muscle, and not over 0.2 p.p.m. in egg.[17-20] Arsenic levels of 1.2 to 4 p.p.m. have been found in livers and 0.1 to 0.9 p.p.m. in muscles of growing pigs fed an arsenical.[6,21-23] This compares with the maximum permitted level of 2.65 p.p.m. of arsenic as spray residue on fruit. The residues mentioned are found if the arsenical is fed up to the time of slaughter. After withdrawal of the arsenical from the feed, tissue residues decrease rapidly. After the required five-day period on a feed free of arsenicals, the arsenic residue in chicken liver was 0.18 to 0.27 p.p.m. and in muscle ranged from undetectable to 0.12 p.p.m.[17,18] Arsenic residue in swine liver decreased to 0.45 to 2 p.p.m., and the residue in muscle decreased to 0.1 to 0.54 p.p.m.[6,21-23]

After several years of use in poultry and swine feeds, the arsenicals were caught (in 1958) between an inflexible law and an uncertain etiology. There is preponderant but not unanimous clinical opinion that skin cancer has occurred in man as a result of long-continued topical application of inorganic arsenic compounds. There is no evidence that organic arsenicals are carcinogenic in any species. But because their use in feeds adds a fraction of a part per million of arsenic to food materials which already contain a trace of arsenic, and because a portion of the fraction of a part per million is presumably inorganic arsenic, and because large quantities of inorganic arsenic applied to the skin for long periods are believed to cause cancer, the Delaney Amendment

prohibits the organic arsenicals in feeds. However, users having prior sanction can continue to use it by virtue of the "grandfather clause." Thus the users with prior sanctions have a sort of monopoly on this alleged method of undermining the public health.

ESTROGENS

Estrogenic hormones rank with arsenicals as the most controversial of the feed additives. The practice of injecting pellets of diethylstilbestrol under the skin of chickens and turkeys was permitted by the Food and Drug Administration from 1947 to 1959. More recently permission was given to use diethylstilbestrol as a feed additive for cattle and dienestrol diacetate as a feed additive for chickens. There was disagreement among the early reports as to whether diethylstilbestrol pellets left a residue of estrogen in chicken tissues. Residues were found by Koch and Heim[24] and by Stob et al.,[25] but not by Jones and Deatherage[26] or Merker et al.[27] Jones and Deatherage used a chemical method; the others assayed with ovariectomized mice. More recently, with an improved mouse assay, Umberger et al.[28] found 20 to 30 parts per billion (p.p.b.) in livers of birds that had received an injection of a diethylstilbestrol pellet or paste, and 35 p.p.b. or more in skin fat. Breast and leg meat contained 2 p.p.b. or less. At the levels indicated by this work a consumer would have to eat 125 to 150 pounds of chicken fat or liver per day in order to get a 2 mg. daily dose of estrogen. This dose of estrogen continued for six days has been found to give a minimal physiological response in man.[29]

The reports mentioned might seem favorable to the continued use of diethylstilbestrol, but it cannot be denied that this compound does cause tumors when administered in large quantities to rats and mice. Therefore, according to present policy, no residue can be permitted. The banning of this compound from poultry production did not have any great impact directly on the poultry industry because it was used in less than 1 per cent of the chickens and turkeys produced. The unfavorable publicity which accompanied the action probably had some impact on consumption of poultry, but this is impossible to evaluate.

Chickens fed dienestrol diacetate at the rate of 32 mg. per pound of feed were found to have no residues in the muscle or fat if the compound was removed from the diet twenty-four hours before slaughter. The chickens did show a level of 3 p.p.b. in the liver.[30] Dienestrol diacetate has not been found to be carcinogenic.

In the controversy over estrogens, some very misleading generalizations have been made regarding the "use of estrogens in feeds." There are important differences among estrogens and among species with respect to estrogen response. Diethylstilbestrol is poorly absorbed from the gut in chickens and turkeys and has never been used commercially in poultry feeds. It is well absorbed from the digestive tract of ruminants and is widely used in beef feeds. Dienestrol diacetate is used in poultry feeds and is well absorbed. In young chickens and turkeys, the estrogens do not consistently improve growth rate or efficiency of conversion of feed. They are, or were, used to induce increased deposition of fat, a result generally considered desirable in young poultry. In cattle and sheep the estrogens increase rate of gain in weight and efficiency of conversion of feed. According to Beeson,[31] "Approximately 75 per cent of the cattle in the feed lots are either implanted or fed stilbestrol. Numerous research studies have shown that either feeding (10 mg. daily) or implanting (36 mg.) of stilbestrol will improve daily gain about 13 per cent and feed efficiency 10 per cent. . . . Stilbestrol is a growth stimulant which increases red meat production but does not increase fat deposition." The economic importance of a practice that increases by 10 per cent the efficiency of three-fourths of the cattle in feed lots in the United States is obvious.

The continued feeding of diethylstilbestrol to cattle is based on the finding of no residue in edible tissues of steers fed 10, 30, or 60 mg. of diethylstilbestrol per steer per day for 180 days.[32] The estrogen was removed from the diet forty-eight hours before slaughter.

The assay method was a mouse test capable of detecting 2 p.p.b. This finding confirmed earlier reports by Perry et al.,[33] Preston et al.[34] and Turner.[35] Other investigators have detected residues by use of the mouse test under some conditions. Wiberg and Stephenson[36] fed steers 10 mg. of diethylstilbestrol per day and found significant residues in muscle when the estrogen was removed from the feed twenty-four hours before slaughter, but not when it was removed forty-eight hours before slaughter. Stob et al.[25,37] found residues in edible tissue of steers after feeding or administering pellets of diethylstilbestrol, or after feeding dienestrol. The feeding level for both compounds was 10 mg. per day, and feeding of the compound was continued up to the time of shipment for slaughter.

Mitchell et al.[38] fed tritium-labeled diethylstilbestrol to a steer at the rate of 10 mg. per day for ten days, then withheld the estrogen for twenty-seven hours, killed the steer and found 0.3 p.p.b. in lean meat, 0.35 p.p.b. in fat, and 9.12 p.p.b. in liver. The quantities found in lean meat and fat would not have been detected by the mouse test.

Estrogen residues have been found with the mouse test[25,39] in lambs treated with diethylstilbestrol pellets.

COCCIDIOSTATS

The coccidiostats are given a separate category because of their importance in poultry production, and because, unlike many of the drugs in the miscellaneous category, they are fed continuously during the life of broiler chickens. Twenty years ago, coccidiosis was the scourge of the commercial broiler industry. It was by far the greatest cause of mortality and of poor quality birds on the market. The available methods of control were ineffective in large flocks, especially during periods of high temperature and humidity. The development of effective coccidiostatic drugs ranks with improved broiler strains, improved feeds, introduction of antibiotics and arsenicals, and development of effective vaccines as one of the primary bases for the modern efficient production of poultry meat.

Published information on residues of some of the coccidiostats is difficult to find. Such information must exist in the files of the manufacturers and the Food and Drug Administration; otherwise permission would not have been granted for use.

In the case of furazolidone Belloff et al.[40] found no residue in tissues or eggs of chickens after feeding this compound at levels five to ten times the usual therapeutic level.

The feeding of nicarbazin to chickens leaves residues of 4,4′-dinitrocarbanilide in the tissues. Forty-eight hours after removal of the drug from the diet, these residues are reduced to traces; four days after removal, the usual chemical methods do not detect the compound in the tissues. After administration of a C^{14}-labeled dose, traces of radioactivity could still be detected in the tissues after four days.[41–44]

Small residues of sulfaquinoxaline are found in livers of chickens after the feeding of this drug, but this fact does not appear to have caused concern.[45]

OTHER COMPOUNDS

Tissue residues of enzymes and vitamins would not seem to constitute any hazard to consumers. The Food and Drug Administration has stated recently that the following information must be provided for any enzyme preparation intended for use in animal feeds: strain of organism used, substrate on which produced, conditions of fermentation, plant facilities used, enzymes found in preparation, and conditions of use in feeds.[46]

A recent news story quotes an opinion of the Food and Drug Administration to the effect that enzymes derived from strains of organisms which are not pathogenic and prepared according to certain specifications are generally recognized as safe and do not require a new drug application or a petition under the Food Additives Amendment.[47]

The economic value of the tranquilizers in animal production has not been firmly established. Their effects on growth of cattle have not been consistent in experimental trials. They are believed to have potential value for reducing stress in cattle during shipping, weaning, dehorning, clipping, castrating, branding,

and parturition.[31] Reserpine appears to reduce the stress of high environmental temperature in laying hens.[48,49] Recently a petition has been filed with the Food and Drug Administration[50] covering tolerances for use of reserpine in chicken and turkey feeds. The petition specifies a tolerance of zero for reserpine in eggs and tissues of chickens and turkeys.

The antioxidant santoquin is a very useful additive for prevention of destruction of the fat-soluble vitamins. It is permitted at a maximum level of 0.015 per cent in poultry feed or in dehydrated forage. There is little accumulation of the chemical in tissues of chickens.[51] Butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT), which are used for stabilization of food fats, are also used to some extent in animal feeds, but they are not very effective as vitamin stabilizers.

Another antioxidant, diphenyl paraphenylenediamine (DPPD), was widely used in poultry feeds until 1956. It had a very low toxicity for several species but after its use in poultry feeds, researchers found that it caused interruption of pregnancy and resorption of litters in rats, and on this basis it was banned.[52] This action in 1956 was taken with a minimum of publicity, although the compound was much more widely used in poultry production than was diethylstilbestrol, and motherhood would seem to be as news worthy as cancer.

Most of the drugs in the miscellaneous category are administered occasionally or intermittently rather than continuously so have less opportunity to build up tissue residues than some of the compounds previously discussed. There is very little published information on residues of these compounds. Presumably such information was submitted to the Food and Drug Administration before permission to use the compounds was granted.

MILK

Not all of the residues in foods of animal origin are residues of feed additives. The presence in milk of detectable levels of antibiotics and insecticides has aroused considerable interest. There are several routes by which antibiotics might get into milk; but, for the most part, residues are traceable to intramammary infusion for control of mastitis. It is estimated that 75 tons of antibiotics are used annually in the treatment of mastitis.[53] Surveys were made of antibiotic residues in the nation's milk supply in 1954, 1955, 1956, and 1959, The percentages of samples containing penicillin in the four respective years were 3.2, 11.6, 5.9, and 3.7.[54] After the first survey, thirty-one authorities on antibiotic therapy, allergy, and pediatrics were polled for their opinions. The consensus was that levels of antibiotic found were not likely to (1) modify the intestinal flora, (2) lead to development of resistant strains of microorganisms, or (3) provoke sensitization of a nonsensitive individual. However, this low level might cause an allergic reaction in a very highly sensitive individual.

There are at least two reports in the literature of allergic reactions apparently traceable to penicillin in dairy products. One described four cases in this country, all of which involved milk from commercial sources.[55] The other involved two cases in England in which both subjects drank milk from their own cows.[56]

Concern over antibiotic residues in milk is felt not only by public health authorities but also by the milk processing industry. Such residues may inactivate the starters used in cheese making. Apparently the key to the problem is to persuade producers to uniformly discard a cow's milk for seventy-two hours after an intramammary infusion of an antibiotic. The Extension Services of the U. S. Department of Agriculture and the states have been carrying on an educational program among producers, especially since 1955.[57] More stringent regulations on labeling of antibiotic preparations for treatment of mastitis have also been adopted, with emphasis on the warning to discard milk seventy-two hours after treatment.

Surveys of insecticide residues in market milk were made by the Food and Drug Administration in 1955 and 1958.[58,59] In the first survey, 62 per cent of 801 samples of market milk contained traces of chlorinated organic insecticides, as determined by a very sensitive fly bioassay. Twenty-one per cent

of the samples, selected for relatively high assay values, were subjected to chromatography, which revealed the presence of BHC, DDT, lindane, DDD, methoxychlor or DDE. Organic phosphate insecticides were not found.

The second series of 936 samples were first subjected to paper chromatography and 2.5 per cent showed 0.1 p.p.m. or more of chlorinated insecticide. The fly bioassay was applied to 168 of these samples; a significant residue was found in 15.5 per cent when a florisil column was used for concentration and in 39.3 per cent when a magnesium oxide column was used. In three herds the source of contamination was traced: to DDT in corn silage in one instance and to careless barn spraying with DDT in the other two.

The Extension Service has developed detailed recommendations as to which insecticides should and should not be used on cattle, in barns, and on various feed crops.[60] With the insecticides, as with the antibiotics, a continuing program of education of milk producers is essential.

COMMENT

I think the following expresses the attitude of the livestock and poultry industries toward additives and residues and the regulation thereof. These industries are proud of the important part they play in making this the best-fed nation in history. Additives and other chemicals which leave residues are not considered a blot upon this record but essential parts of the record which help to make our high standard of nutrition possible. That there is a problem here is still recognized. Additives and residues do pose problems, as do many other aspects of our technological civilization. These problems require the continued attention of everyone, especially of the livestock and poultry industries, but we see no reason for anyone to be alarmed.

It is generally recognized in the livestock and poultry industries that the Food and Drug Administration and state regulatory agencies have played an essential part in the development of our present efficient technology. They have provided intelligent regulation, and we look to them for continuation of this function, for it will become increasingly important as technology becomes increasingly complex. We hope that future policy will be guided by scientific judgment, as in the past, rather than by categorical legislative prohibitions. We hope, also, that when punitive action is necessary, it will be taken promptly and vigorously, but without scaring the public away from good food.

This nation can be fed even better and more economically than it is now, and the possibilities of sharing our food production and processing technology with the rest of the world are limitless.

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Chemical Residues and Additives in Foods of Plant Origin

ROBERT N. GOODMAN, PH.D.*

We have all read accounts of residues of particular chemical compounds in foods or seen reviews of the whole subject of residues and chemical additives. Some were perhaps limited in scope or so broad that many important details and the rationale for the use of these substances were obscured or omitted entirely.

As a horticultural pathologist I have certain obligations to those concerned with the production of plants as food and, to some extent, as fiber. However, as a regular consumer of this produce, I have obligations to myself, to my family and to society regarding our health and welfare. As a researcher in a nonprofit institution, one which is State owned and operated with additional funds made available by the Federal Government, I also have a duty to that segment of the public which includes industries allied with agriculture. Of frequent concern here is the evaluation of chemicals developed for the improvement of crop production.

In my capacity I am able to accomplish this without excessive bias, having nothing to gain by either the success or failure of a given chemical. This is not to say that I am without pressures from the producer who hears of a new wonder chemical to improve yields, or the manufacturer who would like to have a seal of State approval given to his product. I am sure that many members of the medical profession have found themselves in a position where the patient has tried to dictate the prescription and/or where pressure from the manufacturer of a compound were equally great. Fortunately for us both, the preponderance of these compounds accomplish what the manufacturer says they should and in time become a part of our economy, helping to maintain the rate of progress and efficiency of our society. There are of course, certain moral, financial, as well as legal safeguards that limit the number of harmful chemicals that reach the consumer.

One frequently reads or hears comments on the food value and quality of fruits and vegetables of yesteryear. Varieties of apple, like Peewaukee and Ben Davis, which did not have to be sprayed simply because no self-respecting insect or disease would single them out for a meal. What is often forgotten is that these varieties were also not particularly fit for man either. Thus horticulturists have selected and bred varieties which may appear to be botanical monstrosities, but have the eating qualities desired by man. Then we hear, for example, our vegetables have less food value now than in days gone by and, in addition, are covered with harmful chemical pesticides. Time does not permit me to document a refutation to this type of comment. However, I might indicate that standard tomato varieties, such as Rutgers and Marglobe, contain about 15 to 20 mg. ascorbic acid per 100 gm. of fresh weight whereas the content of this vitamin in many new varieties is 50 to 70 mg. per 100 gm.[1] This is an increase of over 200 per cent. Carotene content of the standard sweet potato varieties containing 4 mg. per 100 gm. fresh weight has been raised to 12 mg. per 100 gm. fresh weight, about a

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FIG. 1. Conventional ground sprayer.

FIG. 2. Aerial dusting.

300 per cent increase.[2] In addition, the ascorbic acid content of this vegetable has also been increased, approximately 40 per cent.

If one is permitted to generalize at this point, it would seem that concomitant with an improvement in quality of some of our fruits and vegetables we have had an increase in cultural problems which have in many instances been solved by the use of pesticidal chemicals. The question would of course resolve itself by our return to the inferior fruits and vegetables of former days and, in many instances, varieties that yielded less per acre.

Actually I am not so sure that many of the ills that we cure or prevent today were not with us in earlier times; they just went unnoticed or ignored. They were perhaps accepted as the blight that comes occasionally on potatoes, causing mass migration and famine, or that periodically destroys the pear industry whenever conditions are conducive to the development of the bacterium, or the annual loss of grapes to mildew. There was in most instances no alternative, as the means to protect the plants against these disorders were not at hand.

The following definitive statements indicate the position I have taken with regard to these chemicals. First, there is little doubt in my mind that many of the chemicals used today to combat insects, disease, weed plants, or that alter the metabolism of higher plants, can be toxic to man if ingested, inhaled, or absorbed in sufficiently high concentration. It is well to keep in mind that despite important morphologic and physiologic differences between plants, animals, insects, and microorganisms, their metabolic processes at the cellular level may be essentially the same. Thus a compound which interferes with respiration in one species is likely to do the same in others. Whether or not it does is contingent upon concentration and/or ready access to the site of activity. I should like also to state that the necessary legal safeguards of the public's safety have been, for the most part, already erected. Some may need modification, or require increased personnel to insure their enforcement and to interpret astutely the available research data. I believe, however, that time, patience and logic will obviate these disparities.

The types of equipment used to apply these materials are illustrated in Figures 1 and 2. The pictures illustrate the exposure hazard to which the food producer is subjected. Further, this gives one an appreciation of the quantities of pesticides that can be tolerated by man. Put another way, the laws that protect the spray operator are the same ones that protect the consumer. We should now consider the types of chemicals that are used regularly in the production of fruits and vegetables.

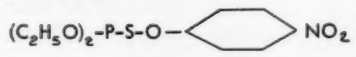
TYPES OF COMPOUNDS

Perhaps the most acutely toxic materials used in crop production are the insecticides. Presented here (Fig. 3) are representative types of the phosphate, chlorinated hydrocarbon and heavy metal compounds. Of these

(1) LEAD ARSENATE

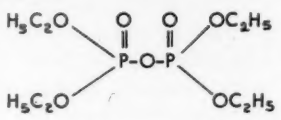
$PbHAsO_4$

(2) PARATHION



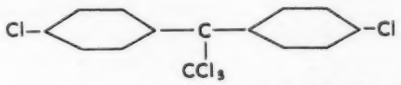
(O, O-Diethyl O-p-nitrophenyl phosphorothioate)

(3) TEPP



(Tetraethyl pyrophosphate)

(4) DDT



(1:1:1-trichloro-2:2-di(p-chlorophenyl) ethane)

(5) DIELDRIN

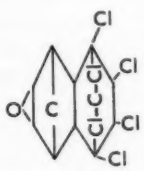


FIG. 3. Typical insecticides.

the phosphate derivatives, e.g., Parathion and TEPP are believed to be the most toxic to warm blooded species, of which man is one. The toxicity of both is due to the inhibition of the cholinesterase enzymes. Depression of these enzymes in both the erythrocyte and plasma fraction of the blood has been documented.[3,4] The enzymes' physiologic function is to catalyze the hydrolysis of choline esters. One of these, acetylcholine, is believed to be the transmitting agent of nerve impulses. Actually the activity of these compounds against the insect, their primary target, is much the same as their effect upon mammals. The literature contains innumerable accounts of fatalities directly assessable to these compounds.[5,6]

It may be said that the effect of the clorinated hydrocarbons, e.g., DDT and dieldrin, is on the central nervous system of the insect. Although there is no clear evidence of their inducing permanent toxic effects in man, 10 mg. per kg. has caused acute illness manifested as headache, nausea, dizziness, and convulsions.[5,7]

The role of herbicides (Fig. 4) or plant hormones in human physiology is at present a highly speculative one. The term carcinogen has been linked to these compounds via l'affaire—cranberry and amino triazole. These substances are used to kill weeds in row crops, as well as dandelions in lawns and poison ivy in back of the house, de-blossom fruit trees, increase fruit "set" (increase the number of fruit developing per cluster), suppress sprouting of onions and potatoes in storage, suppress growth and induce rooting of cuttings. Their place in agriculture becomes more firmly entrenched daily. With such a multitude of effects upon metabolism of plants one should

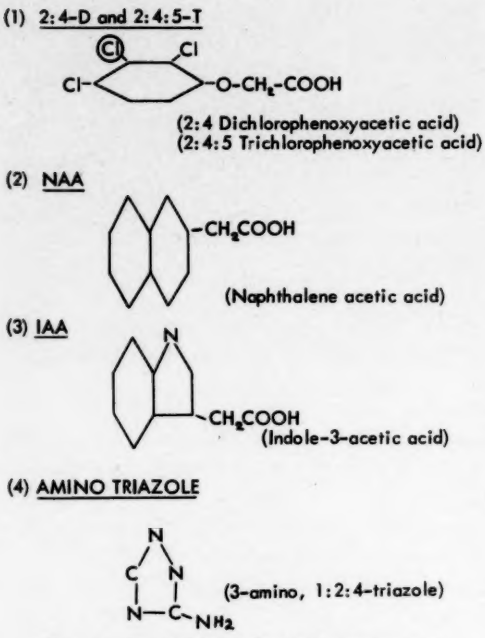


FIG. 4. Typical herbicides (plant hormones).

not wonder that they could deleteriously affect man.

Nitrated and chlorinated phenols are used as herbicides as well as fungicides and it is held by some that their toxic effect upon man may be in their ability to interfere with the production of high energy phosphate compounds which are so essential to cell respiration.

Of the fungicides, perhaps the mercurials, both organic and inorganic ones (e.g., phenyl mercuric acetate and mercuric oxide) are the most hazardous to man (Fig. 5). They seem to combine with and immobilize sulfhydryl groups which are so important in many enzyme reactions. It would seem, however, that the organic fungicides in general have a low order of mammalian toxicity. The bactericides which seem to be the most effective and promising at this time are the antibiotics and we shall dwell at some length on these compounds later on.

Of the final group, the nematocides (Fig. 6) and here we might also include chloropicrin (tear gas), the most hazardous is methyl bromide. These are all applied or at least become active as gases, thus their activity on or in foods of plant origin would be dissipated in a relatively short period of time.

It is apparent now from this brief discussion that the chemical compounds which are used in the production of foods of plant origin are varied and I can assure you almost astronomical in number.

TOXICOLOGY

Of particular concern now, more so than in previous years, is the existence of these compounds within the plant tissues as well as on their external surfaces. It is the realization that some relatively large organic molecules can be absorbed by both the aerial and below ground portions of the plant that has made this problem of residues more disconcerting. In addition, a number of these compounds are actually translocated by the vascular system of the plant. This may not sound particularly startling or unusual to those who are familiar with translocatory systems of members of the animal kingdom, but it is to the plantsman.

Here we find the rationale for the concern for some of the growth regulators which are rapidly absorbed by plant foliage. They have been termed systemic, able to move in the system of the plant. This is equally true for a number of the organic phosphorous insecticides and some antibiotics. The systemicity of these compounds is to a certain extent related to their water solubility. On the other hand the chlorinated hydrocarbons are considered lipoid or fat soluble and their movement into the plant system is limited. One might also ponder the fate of these chemicals, once inside the plant.

We might digress here for a moment to discuss the manner in which these large organic compounds penetrate the cuticular layer of the plant's epidermis. In our laboratory we have obtained almost irrefutable evidence that direct penetration of the cuticular layer of the plant is possible.[8,9] A closer look at the barrier and its morphology and chemical constituents affords the opportunity to visualize how this penetration is effected. There are two hydrophyllic lamellae, consisting of cellulose and pectins which are parallel and wax molecules which

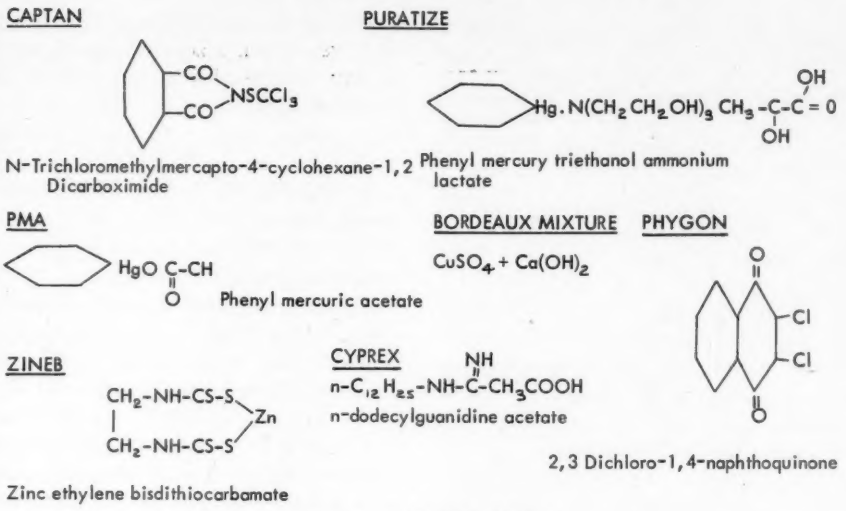


FIG. 5. Typical fungicides.

form in layers radially. Between the two in random orientation is the amorphous polar cutin. The cellulose and pectins are located predominantly in the inner regions of the wall, whereas the waxes are the most numerous towards the outer surface. The cutin contains both hydrophyllic (OH and COOH) and hydrophobic (CH_3) groups, the former are oriented towards the cellulose and the latter towards the wax.[9] This arrangement provides a convenient pathway by which water molecules and water soluble substances may penetrate the epidermis of the plant.

It is apparent now that these chemicals might be located within the plant tissues beyond removal by any washing or leaching procedure. Thus the terms residue and tolerance assume a new dimension. The tolerance for a chemical residue must be calculated in a way which insures its degradation or dilution to a quantity which is nontoxic by the time it reaches the consumer. The fruit growers residue guide assists the grower in keeping his residues within specific limits (Table 1).

Since I am least of all, a toxicologist, I do not believe it is my province to dwell on the matter of safe levels of residues in general. Similarly, regulatory decisions are not mine to make, yet as one concerned with the production of food I should like to describe what has seemed to me and my colleagues to be a logical stepwise procedure in the development of an agricultural chemical. I might add that this method is generally followed for most agricultural chemicals in order to insure effectiveness of the compound for the plantsman, safety to the consuming public as well as financial success to the manufacturer.

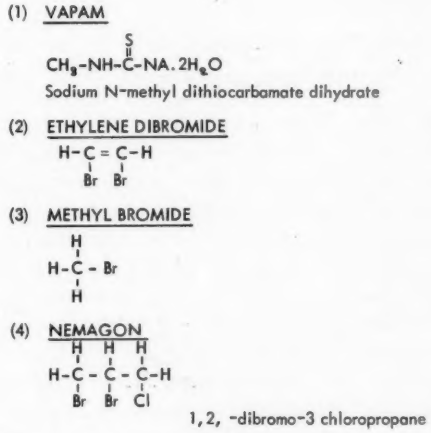


FIG. 6. Typical nematocides.

In the spring of 1952 sprays with two antibiotics, streptomycin and thiolutin, appeared

TABLE I
Fruit Growers' Residue Guide (Partial List)[18]

Compound	Tolerance (μg. per ml.)	Suggested Interval (days)
Bordeaux	Exempt	14
Captan	100	No limit
DDT	0.75	45
Dieldrin	0.1	35
Elgetol 318	0	Dormant only
Lead arsenate	3.5 arsenic 7 combined lead	45
Naphthaleneacetic acid	1	14
Parthion	1	14
Streptomycin	0	No later than 1st cover
TEPP	0	3
Zineb	7	14
2, 4-D	5	7

in our field experiments to reduce the intensity of a bacterial blight of apple and pear. This disease had all but obliterated the pear industry in most areas east of the Rocky mountains. Further it had wreaked sporadic havoc with the Jonathan and other apple varieties, frequently reducing a crop to less than half and limiting production the following year. In 1953 crude spray formulations of streptomycin and Terramycin® at concentrations from 50 to 500 μg. per ml. were evaluated. It was determined from these experiments that 50 to 100 p.p.m. was an effective dosage. At about the same time we developed a simple microbiologic assay which was designed primarily to provide us with information concerning minimum inhibitory concentrations necessary to inhibit the bacterium in plant tissue.[10] With a single spray of streptomycin at 100 p.p.m., a residue in the leaf of 16 p.p.m. was detected after twenty-seven days. At the end of the season, at harvest, the apple fruit was assayed for streptomycin activity, the fruit having been sprayed four times with 100 p.p.m. of the antibiotics. No residue was detected. Although all antibiotics on foods in detectable quanitities were deemed an adulteration within the meaning of Section 402 of the Federal Food and Drug Act, and as such were given a zero tolerance, the absence of any residue cleared these drugs for this specific purpose.

RESIDUES

In 1956 Dr. H. S. Goldberg, of the Microbiology Department of the School of Medicine, and I were awarded a research grant by the National Institute of Health to study "The Public Health Significance of Antibiotic Residues in the Edible Portions of Horticultural Crops." Our initial area of investigation, accomplished with the assistance of J. T. Logue, M.D., was to determine whether or not the spray operators had developed a significant hypersensitivity to streptomycin. In this work a patch test was used. Our results here were negative and induced us to utilize the more sensitive tuberculin-type intradermal skin test using 0.1 ml. streptomycin at 5 μg. per ml. with a suitable control in the opposite arm. A portion of the results from this study has already been reported.[11,12] Approximately 6 per cent of our thirty-two subjects in Missouri, more than fifty in California orchards and 103 agricultural workers in the truck crop area of Florida gave positive reactions. This is approximately the same as was found in the case of a group of nurses[13] and substantially the figure obtained from our group of unexposed control subjects. At present, we believe that streptomycin as used in agricultural sprays is not a particularly allergenic substance.

Streptomycin, oxytetracycline and chlortetracycline were also evaluated in our laboratories in postharvest applications to suppress bacterial soft-rot of leafy vegetables such as spinach and cabbage.[14] In these experiments it was noted that momentary dips of these vegetables into solutions containing the antibiotics significantly delayed the onset of decay. However it was apparent from our bioassays that significant residues of all antibiotics, especially of streptomycin remained in the tissues. Spinach treated with streptomycin at 1,000 μg. per ml. withstood a live steam blanching and rinsing operation and still retained as much as 40 μg. per ml. antibiotics activity.[15] The extreme stability noted for this drug in

fruit and foliage of apple, and reports of its stability in pear trees for a full year, cast some doubt as to the desirability of this antibiotic for extending shelf-life of fresh vegetables.

As residual levels in treated horticultural crops were established these quantities were fed to laboratory animals in treated crops and in drinking water in long-term experiments. Using mice, guinea pigs, and rabbits over a two-year period the following conclusions relative to the emergence of a resistant intestinal flora have been drawn: (1) Following long-term, low-level feeding of streptomycin, 5 to 40 per cent of all coliforms emerge resistant to levels of 10,000 μg. per ml. of this drug. (2) Chlortetracycline did not cause detectable emergence of resistance by coliform bacteria. (3) Blood serum levels of chlortetracycline as a result of long-term, low-level feeding in rabbits resulted in levels that were $^1/_{100}$ attained by a therapeutic dose. (4) It was concluded from these and other studies that public health problems such as emergence of antibiotic resistance, anticipated by widespread nontherapeutic usage of antibiotics are less likely with the tetracycline antibiotics than with streptomycin.[16,17]

It remains now to be seen if similar results will be obtained from long-term, low-level feeding experiments in man. We currently have a study in progress with 50 human volunteer subjects at the Missouri State Penitentiary. Half of the men have been getting 10 mg. of oxytetracycline (Terramycin) and half have been receiving sugar placebos daily since November 1, 1959. Blood and stool samples are obtained at monthly intervals. The blood samples are assayed in my laboratory for oxytetracycline; the stool samples are examined in the Department of Microbiology using the replica plating technic for the emergence of resistance coliforms. In addition, we have been looking for the development of any hypersensitivity to this drug.

What I have tried to convey in this final portion is the general procedure followed in the development of most agricultural chemicals that may find their way into the foods we eat. Our studies did not include the toxicologic tests that usually accompany chemicals in their developmental stages. Obviously, these were not necessary since they had been performed when the drugs were first developed for therapeutic purposes. I might also add that tests with human volunteer subjects are less frequently performed than may be desirable.

I should like to reiterate that the general public has three safeguards for its health and welfare. First, there is Federal legislation that is implemented through the Department of Health, Education and Welfare and the Department of Agriculture. Second, are the efforts of State and Federally endowed research institutions, such as our State Agricultural Experiment Stations and the Agricultural Research Service of the Department and finally, the integrity of our chemical industries. It should be apparent that the reputation of the manufacturer is at stake with each product he endeavors to place before the public.

ACKNOWLEDGMENT

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Industry Problems in Developing Intentional Food Additives

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FOOD additive problems affect many diverse areas of American industry. Some companies, like the chemical manufacturer and flavor compounder, may prepare substances intended for deliberate addition to food. Others, like the package fabricator, the machinery manufacturer, the pesticide formulator and the petroleum refiner, may be more concerned with substances which unavoidably get into food. The food processor and feed manufacturer are users of food additives.

Many companies with related interests have approached food additive problems through their trade associations; it has been estimated that at the present time there are between 50 and 100 different trade associations to whom food additive developments are of concern. It is obvious, therefore, with such a range of interests, that there is no over-all industry attitude or position of food additive matters. I shall provide you with one viewpoint on a few food additive problems, a viewpoint likely to be shared by many in industry, but certainly not by all. It must be considered a personal viewpoint and not necessarily one shared by my company or by the chemical industry.

I shall use the term food additive in the sense defined by the Food Protection Committee of the National Research Council, namely "A substance or a mixture of substances, other than a basic foodstuff, which is present in food as a result of any aspect of production, processing, storage or packaging." I must define this term because the legal definition of food additive, under the Food Additives Amendment of 1958, is a much more restricted designation from which most additives in everyday use are excluded because they are generally recognized as safe.

It is convenient to divide food additives into two classes. When they are introduced to preserve or to improve the quality of the product, they are known as intentional additives, because they are purposely added to serve a specific need. In contrast, other food additives are known as incidental additives. They include substances like pesticides, which are required for the production of crops and which may remain in small quantities in the food. Because my experience has been with intentional additives, I will limit my remarks largely to problems with this type of product.

INTENTIONAL ADDITIVES

Since intentional additives are introduced into food for specific purposes, they may be classified in terms of what they do. Possibly the most extensive and authoritative list of intentional food additives is that compiled by the Food Protection Committee of the National Research Council. It is entitled, "The Use of Chemical Additives in Food Processes." This booklet was first published in 1956 and is currently being brought up to date through revision.

An important group of intentional additives are the nutrients, the vitamins, minerals and amino acids which may be added to certain staple foods to restore nutrients lost in processing and to otherwise eliminate general dietary deficiencies. Iodized salt, vitamin D fortified milk, enriched cereals and grains, and vitamin A fortified margarine are illustrations of usage of intentional additives which are

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not only endorsed by leading nutritionists but in many states are required by law.

For those who must or who prefer to minimize sugar consumption in their diets, a variety of foods are commercially available containing saccharin or cyclamate, non-nutritive sweetener additives. Food colors and flavors are two classes of additives whose uses are obvious. Incidentally, in terms of numbers of different compounds, flavor chemicals exceed all other intentional additives combined. Artificial food flavors are generally mixtures of many different compounds, carefully selected and blended. Such blends are, however, still simpler than natural flavors. For example, natural orange and grape flavors have each been shown to consist of at least several dozen different compounds.

Emulsifiers, surface active agents, sequestering agents, maturing agents, buffers, acids and alkalis are other classes of intentional additives of particular importance to the food processor. They permit him to compensate for natural variations in his raw materials such as hardness of water, acidity of cream and baking properties of flour, and enable him to produce foods which are consistent in appearance and which remain physically stable.

Much of the food we purchase today is preserved from deterioration by some sort of physical treatment, such as cooking, canning, bottling, pasteurizing, refrigerating, freezing or dehydration. In some cases, intentional additives are used for similar purposes. Antioxidants, for example, prevent rancidity in fatty foods. Antimycotics prevent molding of bread, citrus fruits and cheese. Vitamin C is used to prevent darkening and flavor changes in freshly cut and frozen fruit and in peeled potatoes. Antibiotics can extend the shelf life of refrigerated poultry and fish.

The foregoing are just a few examples of what we mean by intentional food additives. Keep in mind that these are substances deliberately added to foods for specific purposes, to improve nutritional quality, attractiveness, uniformity, keeping quality, ease of preparation and cost. Intentional food additives must benefit the consumer in either a direct or indirect way. Otherwise, there is no justification for their use.

Intentional food additives and adulterants are sometimes confused. The purpose of an adulterant in this connection is deception, to make a food look better than it really is, to disguise deterioration or decomposition, to substitute for a valuable ingredient, to cheapen. The function of an adulterant is almost precisely the opposite of an intentional food additive, this is, deceiving or degrading as compared to improving. Adulterants have no place in our food supply and we have long had laws prohibiting their use.

Intentional food additives are absolutely essential to our life today. Without them, our diet would be nutritionally inadequate, restricted, monotonous and expensive. Without them, familiar foods like bread, processed fruit, soft drinks, cheese, margarine, canned vegetables, ham, sausage, candy and ice cream would be eliminated from our meals. We would be left with a few fruits, vegetables and meats to be eaten without benefit of sugar, salt, butter or spices.

On the basis of our past experience, it seems obvious that our use of intentional food additives in the future will increase.

The United States today has the most nutritious and wholesome food supply in its history and in the world. In the future, it seems likely that we will continue the same conservative approach to the use of nutritional adjuncts that we have followed in the past. However, with our increasing knowledge of nutritional requirements and deficiency diseases, some modification in vitamin content, protein composition, and particularly the fat composition of our diet, may be desirable through food selection and food fortification practices.

Important reductions in food spoilage losses have been achieved through the use of pesticides, refrigeration, freezing, rapid transportation and chemical preservatives. Much still remains to be accomplished, for food spoilage is still a multibillion dollar challenge and a luxury that we cannot continue indefinitely to afford with our expanding population.

Convenience foods, unknown a generation ago, are now readily available to take the drudgery and uncertainty out of home cooking.

Additives made some of these developments practical, and promise exciting possibilities for the future.

Another development of great importance to the consumer is in food quality. The wormy apple, the stale cracker, the rancid peanut have almost disappeared from the scene. The tough steak may be the next to go. Intentional food additives have made, and will continue to make, important contributions to the availability of foods of consistently high quality in your future.

These are just a few of the areas in which food additives will perform functions of increasing importance in the years ahead. I have not attempted to speculate on possibilities which have no basis in commercial reality today, but it must be obvious that much effort is being devoted to exploring entirely new fields of application for additives to food. Certainly, if there is one thing which we in industry do not lack, it is in ideas of ways in which our foods can be improved in quality, in availability and in cost, through the proper use of food additives.

THE FOOD ADDITIVES AMENDMENT

The Federal Food Drug and Cosmetic Act was amended in 1958 through the enactment of the Food Additives Amendment. This amendment is a major piece of legislation which provides extensive controls over the use of intentional and incidental food additives.

The legislation was enacted with two purposes in mind according to the Committee on Interstate and Foreign Commerce of the House of Representatives: (1) to protect the health of consumers by requiring manufacturers of food additives and food processors to pretest any potentially unsafe substances which are to be added to food; and (2) to advance food technology by permitting the use of food additives at safe levels.

These are noble objectives. They are supported by industry. Specific legislation to accomplish these objectives was advocated by industry groups for several years prior to enactment of the present law. To the extent that the Food Additives Amendment continues to fulfill these objectives, I am sure it will continue to be regarded by industry as a good law.

Enactment of the Food Additives Amendment has created a number of problems for industry because of its scope and its newness. It has raised questions concerning the status of hundreds of substances commonly used in or on our foods for years. It has involved the products of suppliers with no background of experience in dealing with food regulations and with the Food and Drug Administration. It has threatened formulators with exposure of their trade secrets.

Under such conditions, it is hardly surprising that there has been much misunderstanding on the part of many companies concerning the Food Additives Amendment and its interpretation. Even with companies considered to be experienced in dealing with food law, there has been widespread frustration because of regulatory delays.

Although these are all problems which time must solve, I do not wish to minimize their importance to industry. The Food and Drug Administration has done a tremendous job in the time available to them in administering the Food Additives Amendment, but there is serious concern as to whether there is enough time before March 6, 1961, when present temporary extensions expire, to permit both the Food and Drug Administration and industry to deal properly in deciding the status of hundreds of existing food additives. An amendment to the law to permit further extensions for food additives when there is no danger to public health has been widely advocated by industry spokesmen as a means of avoiding a potentially chaotic situation.

Our year and a half of experience under the Food Additives Amendment has served to reveal many other problems which time can only aggravate if corrective steps are not taken. Some of these problems are of particular concern to the scientist because they seem to represent instances in which the ability to act and to regulate on the basis of right scientific judgment has been sacrificed to administrative convenience and political emotion.

The prime example of this is, of course, the

so-called Delaney clause which prohibits approval for use in food of any substance found to induce cancer when ingested by man or animal. This is the clause which has helped bring to a practical standstill progress in animal feed additives and has already caused two leading pharmaceutical companies to curtail severely their research programs in this area.

Various arguments have been raised against the Delaney clause. These are in essence based on the fact that experienced judgment as to whether a hazard exists in the use of an additive has been replaced by an arbitrary requirement permitting no interpretation. I should like to emphasize that in the widespread opposition expressed to the Delaney clause, there has at no time been any suggestion that harmful carcinogens be permitted in the human diet. Although many believe the Delaney clause should be dropped entirely, a simple qualification by specifying that to be prohibited from use the additive must be found to induce cancer when ingested by man or animal "in amounts and under conditions reasonably related to the intended use" would remove most industry objections.

Another vexation to the scientist is defining the practical equivalent of zero. You may be aware that a food packaging component is not a food additive if it does not migrate into the food. Certain pesticides are approved on the basis that there is no residue in the edible part of the food. A drug for animal use may be approved on the basis that it leaves no residue in edible tissue. All these applications pose the question of how one can demonstrate that a substance is not present. It is, of course, quite possible for the analytical chemist to develop methods capable of detecting one part of additive in one million parts of food and by such a procedure he may show that no detectable quantity of additive is present. By more refined techniques he might show no detectable additive in 10 million parts, in 100 million parts, or even a billion parts of food. The law gives the analyst no guidance as to the practical equivalent of zero that it will accept.

Conversely, if there is an effective food additive regulation involving a zero residue specification developed on the basis of an analytical method sensitive to one part in a million, will this approval hold in the event of the development of a more sensitive procedure which might show, for example, a detectable residue of one part in 100 million? It is probably fair to say that at the present time, anyone operating under a zero tolerance requirement is vulnerable to progress in analytical chemistry.

In order to resolve problems of this sort, we badly need principles, based either on scientific testing or on experience, which will guide both industry and the regulatory agencies in establishing limits for additive residues sufficiently small to be negligible insofar as safety is concerned. This will in turn enable the analyst to determine the sensitivity of the methods he must develop in order to detect whether a biologically significant residue of an additive is present in a food.

Other areas in the Food Additives Amendment could be cited in which there is an apparent conflict between scientific judgment and the regulation or in which such a conflict could develop if the regulation were interpreted very narrowly. It would seem highly desirable to both government and industry to resolve such situations and in this connection the Food Protection Committee is playing an important role.

THE FOOD PROTECTION COMMITTEE

The Food Protection Committee operates under the Food and Nutrition Board of the National Research Council, National Academy of Sciences. It was formed about ten years ago as an advisory group, its primary purpose being to provide critical evaluation of information concerning the use of chemical additives in foods for the Council and encouragement of the food industry and as a guidance for public agencies.

The Food Protection Committee membership is drawn entirely from universities. However, through its panels, the Committee maintains effective liaison with governmental agencies, trade and scientific associations, and interested industries.

During the almost ten years of its existence, the Food Protection Committee has issued several authoritative reports on food additive problems including one on "Insignificant Levels of Chemical Additives in Food," another on "Principles and Procedures for Evaluating the Safety of Food Additives," and a third on "Problems in the Evaluation of Carcinogenic Hazard from the Use of Food Additives." In preparing these reports, the Food Protection Committee has had available to it the experience and advice of the most outstanding and knowledgable scientists in this country.

The regulations of the Food Additives Amendment provide that in matters of the adequacy of the methods employed to demonstrate safety for the proposed use, the Food and Drug Administration will be guided by the current publications of the Food Protection Committee.

Many of us in industry believe that a more thorough acceptance of the broad principles expressed in the reports of the Food Protection Committee in the regulation and enforcement of the Food Additives Amendment would do much to resolve many of the conflicts that described herein.

Thus far, some of the typical immediate problems resulting from passage of the Food Additives Amendment have been discussed. Let us consider now what is happening to our long range research programs from which new food additives for 1965 and 1970 should be coming.

PROBLEMS OF INDUSTRY

Justifying the effort to develop a new food additive is at best difficult. Here are some of the problems.

(1) We must find an effective additive, sometimes a very difficult undertaking, always an uncertain one. In an additive development our company undertook a few years ago, more than 3,000 compounds were prepared and tested in one application before we found one that met our needs for effectiveness.

(2) The additive must be economical and acceptable for food use in terms of color, flavor, taste, physical properties, ease of use, etc., a further limiting factor.

(3) The additive must be safe. It is a fairly simple matter to detect gross toxicity and eliminate a highly toxic product from a testing program at an early stage. Less obvious toxicity takes longer to spot. The possibility that an effective additive may be toxic further decreases chances of successful research; occasionally much time and effort is invested in a product before it becomes obvious that it is unacceptable.

The difficulties and expense of food additive research, the severe requirements for an acceptable product, and the long time required to clear a product for commercial use all require that we restrict our objectives to markets of considerable size. Some companies have indicated that a market of one million dollars per year was the minimum they could consider in justifying a research program. Although the value of the food produced in this country is very large, it should be kept in mind that intentional food additives are often used at extremely low concentrations. Research on certain types of intentional food additives such as new flavors and new food colors is likely to be greatly reduced for such reasons.

Enactment of the Food Additives Amendment can be expected to make the field of food additive research distinctly less attractive to industry for several reasons.

(1) The amendment has introduced a major new requirement, that for a suitable analytical method for determining the additive in food, which will increase the cost of the development appreciably.

(2) The requirement that a food additive petition be filed and approved will probably delay the marketing of a new food additive for at least an additional six months.

(3) The announcement, by publication in the Federal Register, that a company has petitioned for approval of a new food additive can be expected to precede the approval of the petition by at least five months. This is ample time for competition to develop a manufacturing process, erect a production unit and be ready to market the additive as soon as the approved regulation issues. Unlike the approval procedure for new drugs, which is

sometimes cited as a parallel regulation, the Food Additives Amendment deliberately discloses the development of the company that has discovered the additive, borne the expense of developing analytical and safety information, and prepared the petition. The Food Additives Amendment goes far in destroying the traditional privilege given to enterprising companies under our American system of being able to proceed in confidence with a new development and to achieve a competitive advantage by being first on the market.

(4) Finally, it should be emphasized that a company which believes it has performed a thorough and competent job in preparing a Food Additive Petition and in supplying the volumes of data required, has a rather poor chance for regulatory approval. In a recent address by the Deputy Commissioner of the Food and Drug Administration, it was indicated that as of mid-March 1960, petitions withdrawn because of incomplete information outnumbered those approved by 50 to 10. Such experience can only further decrease the attractiveness of food additives as a field for research.

These are but a few of the problems facing industry under the Food Additives Amendment. They not merely affect companies and products brought under the jurisdiction of the Food and Drug Administration for the first time but also those of whom it used to be said that the Food Additives Amendment would simply formalize the procedure they had been following voluntarily for years. They are problems affecting those who would work for a better and more abundant food supply for tomorrow.

On the basis of our past experience, it seems obvious that an increased usage of intentional food additives will be needed in the future not only to meet the food requirements of our expanding population but also to enable us to continue to enjoy the freedom of an economy in which today the efforts of only 13 per cent of our people need to be devoted to food production. One of our great sources of strength lies in the effort we can direct toward pursuits other than those concerned with producing our food and maintaining our existence. It would be a tragedy if, by our decisions today, we deny this source of strength to the America of tomorrow.

The public must be assured that its food supply and its permitted food additives are safe. The public must also be assured of its rights to the benefits resulting from the proper use of food additives not only today, but also in the future. How to reconcile these objectives without compromise is a challenge to scientists in industry, in the university and in government. Certainly, a better understanding, appreciation and respect for our mutual problems is an essential first step for I cannot concede that there should be any basic conflict in our philosophies.

Government Problems in Dealing with Additives and Residues in Food

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ABOUT a year and a half ago, after it had been publicly announced that I had been appointed to a newly-created position with the Food and Drug Administration, I received a number of letters and comments from friends and acquaintances. One man—a former university professor of pharmacology who had recently been operating a drug research and testing laboratory—wrote: "So you are going with my old enemy, the Food and Drug Administration. Congratulations and condolences." Another friend—a member of a family with unusual and somewhat unorthodox views on drugs and health—said: "I wish you success. It is about time that the Food and Drug Administration did something about protecting the public."

These two comments represent extreme views about the enforcement activities of the Food and Drug Administration. One group seems to believe that it is an unreasonable ogre waiting for a chance to jump on and destroy legitimate industry; the other, that it is a complacent bureaucracy which does nothing to protect consumers from dangerous substances in foods and drugs but is, in fact, a tool of big business. Because these extreme and contradictory views are held by small groups of people it may be concluded that the Food and Drug Administration is probably taking a sane, reasonable course—protecting the public against dangerous substances in foods, drugs, and cosmetics without unduly harassing proper agricultural and industrial activities. When an agency is criticized by both extremes it is possible that it is doing its job well. This may, in fact, explain why the Food and Drug Administration is the neglected stepchild of the federal government in the opinion of some people. We are not radical enough to gain the support of the rabid food faddists, but too insistent upon a reasonable observance of the law to have the wholehearted support of some segments of the regulated industries.

The policies and viewpoints of the Department of Health, Education and Welfare and of the Food and Drug Administration are contained in the mandate of laws as passed by the Congress. To be sure, at times the Department of Health, Education and Welfare and the Food and Drug Administration may have some influence on the nature of the bills submitted to the Congress and adopted by it, but in the final analysis Food and Drug Administration must be guided by federal law as written by the Congress and as interpreted by the courts. We can take only such actions as are specifically authorized; to fail to enforce the law as effectively as possible would be a neglect of duty.

In discussing a subject of this kind there is a temptation for an official of the Food and Drug Administration to stick closely to the wording of laws and regulations. This avoids the possibility that he will be misunderstood by a person concerned with the strictly legal aspects of the matter. But the legal phraseology would not be very meaningful to you, whose training has been in science rather than in law. Consequently I shall attempt to explain, in common English words, the meaning of the laws and regulations as currently

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interpreted by the courts and by the Food and Drug Administration.

The forerunner of the present Food, Drug and Cosmetic Law was passed more than fifty years ago, but during that half-century numerous changes have been made in the law. These have been required because of changes in agricultural practices, food technology and dietary habits of the American people. When the first general food law was passed by the Congress in 1906, food technology was in its infancy. Most of the food going into the home was in the raw, unprocessed form. Prepackaged foods were relatively unknown; cheese was cut from the "wheel," flour was weighed out of a sack or barrel and sold in bulk. Processed fruits and vegetables in cans were common, but the home preserving of fruit in glass jars was likewise common. Condensed and evaporated milk were common articles of commerce but dried milk powder was unheard of. Market milk was often dipped out of a bulk container into the customer's own pail. Much of the food of the average family was either produced on its own farm or backyard garden or was obtained locally from nearby farms.

At that time there was no great concern about spray residues of pesticides on food. Agricultural poisons to control pesticides were almost exclusively inorganic compounds such as copper, arsenic, lead, and fluorine or natural poisons such as nicotine and rotenone.

But poisons *were* being used in food. Dr. Harvey W. Wiley, the father of the first pure food law, was a zealous campaigner against preservatives in food. He organized his "poison squad"—volunteers who became human guinea pigs to test the chemical preservatives of that day. Among the substances being used were formaldehyde, boric acid and benzoic acid. Dr. Wiley was thus concerned about the problem we are discussing today—chemicals in food.

Dr. Mahoney (see page 277), used the term "food additive" to include any foreign chemical which finds its way into food, whether intentionally added to produce a flavor or color, or to produce a physical effect; or whether it becomes a residue in food from pesticide treatment of the crop. In the legal sense, as defined by the Food Additive Amendment of 1958, the term "food additive" has a much more restricted meaning. It does not include pesticides on raw agricultural products, coal-tar colors, substances generally recognized as safe and substances permitted by prior sanctions. This difference in usage of the term can lead to confusion. They can be distinguished in writing by using capital letters to designate the legal term "Food Additive," and using lower-case letters for the more general term. In the spoken word, however, there is no easy means of indicating capital letters. It might be well for me to outline the various types of additives, residues, and contaminants in foods from the *legal* standpoint.

PESTICIDES

Pesticide residues on raw agricultural products are controlled under terms of the Pesticide Act of 1954, the so-called Miller Amendment to the Food, Drug and Cosmetic Act. A manufacturer who wishes to introduce a new insect-killer or other agricultural poison which may become a contaminant in food, registers the substance with the Department of Agriculture. If that Department, upon examining the information submitted, sees the likelihood of the substance appearing as a residue or contaminant in food, the matter is referred to the Food and Drug Administration. The manufacturer may petition the Food and Drug Administration for a residue tolerance for the substance on raw agricultural products. If the Department of Health, Education and Welfare, that is, the Food and Drug Administration, finds that the substance is harmless to man and animals, its use may be permitted without a tolerance being imposed. If it is toxic to man or animals and if the data submitted by the manufacturer show that its use will result in small, but safe, levels of residue, the Secretary of Health, Education and Welfare is authorized to issue a regulation establishing tolerances on designated food and feed products. It is then the responsibility of the Food and Drug Administration to see that these tolerances are not exceeded.

Before a pesticide manufacturer submits a request to the Department of Agriculture for

a registration of his label he has the substance tested under actual farm usage. Such experiments may be carried out on the manufacturer's own experimental farm. Or, as many of you know since you have participated in such studies, the tests are often carried out by state agricultural experiment stations in cooperation with the manufacturer. Such testing will show the necessary level of use to obtain the desired result and will reveal the desired time and frequency of application. This information, together with analytical methods and the results of toxicological tests, is submitted in his petition to the Food and Drug Administration for a tolerance. The Food and Drug Administration must be convinced that the proposed tolerance level is safe before issuing a regulation permitting use of the substance.

Illegal residues of pesticides on food or feed crops may result if the label instructions as to dosage and method of application are disregarded or ignored. This means that persons who use agricultural chemicals must be able to read, must understand what they read, and must be willing and intelligent enough to follow the instructions. There was a time in certain areas of the world when the production of food crops was deligated to illiterate peons. Even in early American years many farmers lacked formal education. But those days are gone forever. Many agricultural chemicals are too potent to be placed in the hands of persons who cannot read and therefore cannot follow the label instructions.

Even intelligent and educated persons have occasional lapses when they fail to understand the real significance of instructions. To be followed, instructions must be in terms which the person can understand. Furthermore, instructions for use of agricultural chemicals should be placed on labels where they can be readily seen. A colleague recently showed me a label which he had cut from a 100 pound sack of poultry feed. The feed contained a drug which had possibilities for harm if the instructions for use were not carefully followed. But the warnings were printed on the *back* of the label, and the label had been stitched to the burlap bag longitudinally and down the middle. The warning instructions could not be read until the label was removed.

FOOD COLORS

Synthetic so-called coal tar colors are not covered by the Food Additive Amendment of 1958 but are still controlled by the provisions of the 1938 Food, Drug and Cosmetic Law. When the 1938 law was passed by the Congress it was believed that the coal-tar colors in use were completely harmless. The law provided, therefore, that certified colors shall be ". . .harmless and suitable for use." Later it was found that some of those colors were not, in fact, harmless when fed to laboratory animals in relatively large amounts. Under the provisions of the law, therefore, the Food and Drug Administration was required to remove such colors from the certifiable list. The 1938 law does not provide for setting tolerances for colors which are harmful in large amounts but which would be safe under conditions of use. There are now bills before Congress which, when passed, will permit the Food and Drug Administration to set tolerances for colors which are toxic in large amounts but which would be harmless in the amounts used.*

PROVISIONS OF THE FOOD ADDITIVE AMENDMENT

The Food Additive Amendment of 1938 to the Food, Drug and Cosmetic Law classifies several types of contaminants or residues in foods. They are (1) substances generally regarded as safe; (2) substances with prior sanction for use; and (3) food additives. As I indicated earlier, coal tar colors and pesticides on raw agricultural products are excluded from the provisions of the law. The term "Food Additive" is defined, legally, in a very special sense.

Substances Generally Recognized as Safe

The Amendment excludes from its provisions a substance which is generally recognized as safe ". . .among experts qualified by scientific training and experience to evaluate its safety." The law does not state how many experts must recognize a substance to be safe,

* The Color Additive Amendments became Public Law 86-618 on July 12, 1960.

or whether a majority of such experts must so recognize it, or whether *all* such experts must accept its safety. Suppose that a substance falls into such a highly specialized group of compounds that only five persons in the United States have any detailed and intimate knowledge about it; if all five of these men believe it to be safe, is this general recognition of safety? Or, suppose that 100 experts are questioned about a specific substance; ninety-nine of them express the opinion that it is safe, but one who has some special information unavailable to the other ninety-nine states that the substance is unsafe. These are some of the kinds of problems faced by the Food and Drug Administration in the evaluation of substances "generally recognized as safe." Nevertheless, after some lost time, several hundred substances have been classified in this group and lists of them published in the Federal Register.

One point is of special interest in connection with safety: the Amendment states (Sec. 201 (t)): "The term 'safe,' as used in paragraph (s) of this section and in section 409, has reference to the health of *man or animal*" (emphasis supplied).

"Food Additives"

Food Additives, in the legal technical sense, are substances added to or which become components of food which are *not:* pesticides on raw agricultural products, coal tar colors, substances with prior sanction under the "grandfather clause" of the law or substances generally recognized as safe. It is evident that to be classified legally as a Food Additive it is either not entirely safe, or it has not been adequately tested or in use long enough that its safety is recognized by scientists qualified to evaluate such safety. The Amendment gives the Secretary authority to exempt from the tolerance provisions of the law, substances which he deems to be safe on the basis of information supplied in a Petition. Or, if a substance to be used in food processing, manufacture, packaging or storage will not yield residues in the finished food products, it may likewise be exempt from the provisions of the law.

For all other additives, approval for use must be obtained through Petition to the Secretary of Health, Education and Welfare. Such a petition must include information on the chemical nature of the material, mode of manufacture, toxicological data on safety, practicable method of analysis for the additive and a statement of intended conditions of use. If the Secretary finds that the use of the additive will be safe, will not lead to deception or misbranding, and will effect its intended use without exceeding necessary safe tolerances, he may issue an order permitting its use.

Food Additives have sometimes been classified as (1) incidental, and (2) intentional. The *incidental* additives include such substances as traces of packaging material which migrate into the food, lubricants from processing or packaging machinery, and metals or other components of processing machinery which are dissolved or abraded into the food during manufacture.

The *intentional* additives are those which are added purposefully to confer some physical, chemical or other effect upon the food. They may include enzymes, antioxidants, emulsifiers, antifoaming agents, dough conditioners, mold-inhibitors, flavors and a score of others.

COMMENTS

These general considerations may seem either confusing, or redundant, depending upon the extent of your background information. The public is well aware of a number of controversial problems facing the enforcement program of the Food and Drug Administration under the Food Additive Amendment. You have all heard of the aminotriazole-cranberry situation under the Pesticide Act, the diethylstilbestrol-poultry controversy, and the general problem of the policy of carcinogenic substances. The dairy industry is worried about antibiotics and pesticides in milk and dairy products.

Regarding the last mentioned subject, the Food and Drug Administration has been concerned about pesticides and antibiotics in milk for several years. Surveys of market milk were made in 1955, 1956 and again in 1958. Between the 1956 and the 1958 sampling pro-

grams, an intense educational campaign was instituted in cooperation with other governmental and nongovernmental agencies. That educational campaign sharply reduced the number of samples containing these contaminants, but did not reduce them to zero. The Food and Drug Administration therefore announced its intention to sample milks and milk products in its enforcement program, and, if any were found illegal, to take regulatory action. News media have carried the information recently that shipments of evaporated milk produced in California have been seized because of illegal contamination with chlorinated hydrocarbon pesticides.

The problem of antibiotics in fluid milk poses two problems, one of economics, the other of health. Antibiotics in milk going to cheese manufacturers interfere with the action of bacterial starter cultures. This is a serious economic problem. On the health side, many people are sensitive to traces of penicillin, and may suffer severe illness or even death from a small trace of the drug. There is no justification for antibiotics getting into milk; the milk producer can control this by discarding milk from cows given penicillin for treatment of mastitis by following the directions for use; that is, by discarding the milk for seventy-two hours after treatment. Milk from an infected udder is not fit for use whether it contains an antibiotic or not, so there are two reasons for destroying such milk. Penicillin used in the treatment of other bovine diseases, such as that given by parenteral injection, can be kept out of market milk by discarding the milk for an appropriate length of time. The Food and Drug administration has recently proposed appropriate label warnings for antibiotic preparations given intramuscularly or intravenously.

The matter of keeping pesticides out of milk and dairy products is not quite as simple as it is for penicillin, since milk producers often buy feed for their stock. However, the producer can control this by purchasing feed only from reliable sources, known to supply pesticide-free stocks. The producer may also wish to insist upon a guarantee that the feed or forage he purchases does not contain any non-permitted pesticide residues.

No one in government wishes to stifle the proper use of chemicals in food production and processing. None of us wants to go back to the days of moldy bread, wormy apples, expensive steak and tough poultry. Yet that is what would happen if the use of agricultural chemicals were unduly restricted. We all have a stake in this—farmers, industry, consumers, the government—for we are all consumers. In government our concern is that *potentially* dangerous substances are properly used.

Medical View of Additives and Residues in Foods

J. M. Coon, M.D., PH.D.*

From the medical viewpoint our main concern regarding chemicals to which the human population may be unavoidably exposed in its food, water and air environment is the potential health hazard involved in such an exposure which may continue throughout life. Some general biological and medical considerations of chemicals in that part of the environment related to food will be discussed herein.

Since the procedures recommended and widely used for evaluating the safety of chemicals which become additives or residues in our food supply have been well described elsewhere,[1-4] these will not be outlined here. Some thought, however, will be given to the limitations and the need for improvement in our approach to the problems which confront us.

In the present state of our knowledge the medical view of additives and residues in foods must be a highly conservative one. Most of the population of our country is the captive consumer of the nation's food supply, and any undue relaxation of our vigilance could result in widespread injury to health which might be very difficult to relate to any specific substance in our diet. Therefore, the calculated risk, or the ratio of the potential hazard to expected benefit, as these factors may be judged by qualified experts, must be disappearingly small in the case of additives and residues as compared to the hazard: benefit ratio which is found acceptable for drugs used in the treatment of disease. In the latter case we have many approved drugs, the recommended dose of which will cause undesirable or severe reactions in some of those taking them. Some drugs may even be lethal in a few hypersensitive patients given the recommended dose. But in the field of medicine it is permissible to use a dangerous drug against a more dangerous disease. It is clear why we cannot have a similar situation in the case of additives and residues in foods. Here we are starting with a healthy person and we must keep him healthy. Ideally, we should avoid even the remotest possibility of his being injured by additives and residues in his food.

Fortunately, due caution is being exercised in this regard. Some people believe that we are being overly cautious in some respects, for example in allowing *no amount* of certain pesticides to appear in our milk supply. This overcautiousness, if that be what it is, is forced upon us by our ignorance. For example, we do not know how much DDT can be tolerated in the diet through sickness and health from the cradle to the grave. Even the qualified experts disagree on points of this kind. Only the future will tell where we have been overcautious, or perhaps where we have let something harmful filter through our rigid guard.

In any case, we are playing it as safe as we know how while keeping our heads level at the same time. Much thought, research, and control is being brought to bear to avoid injury to public health through additives and residues, and this is being accomplished unmotivated by any evidence that these substances, when used in foods as intended, have ever seriously affected the public health adversely, although many have been in use for several decades. The situation is much better

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in hand than was the case with drugs in 1937. In that year a drug company dissolved sulfanilamide in diethylene glycol and put the preparation on the market without testing it on animals, and even without becoming informed of the existing knowledge of the toxicity of diethylene glycol. About seventy-five people died of diethylene glycol poisoning before the preparation was withdrawn from the market.[5] No such specific episode or no series of minor episodes precipitated our present legal controls of additives and residues. Our present activities in this field constitute the practice of true preventive medicine.

Since in many cases we are not able, with complete assurance, to pronounce a substance fit or unfit for additive service on the basis of factual experimental observations, we find it necessary to do so on the basis of judgments formulated from "educated" extrapolation of the laboratory data obtained on animals. We frequently refer to our judgment of how much of a given substance can be safely present in the human diet as an "educated guess." The educated guessers are the scientists who are familiar with the accumulated knowledge of toxicology and its methodology, and they make their judgments in that light and in the light of the data specific to the agent under consideration.

Opposed to the educated guessers are the uneducated ones, who write for public consumption under such titles as "The Poison on Your Dinner Table," "Modern Diet in U. S. Loaded with Poison" and "The Poison in Your Food." The latter, a recently published book[6] and the crowning glory of all, will certainly be the winner of the Pulitzer prize for poisonous literature. The doctor who is confronted with a patient poisoned by such print may well have on his hands a case for referral to the psychiatrist or any of a number of specialists in the psychosomatic diseases.

Public reaction to these fear-provoking articles may result in distortion of the diet and starvation of the purse all out of proportion to what is considered, respectively, good nutrition and good economy. More people may become food faddists looking for natural, untreated, unprocessed, unmanufactured, "unadditivated" foods.

Many of the uneducated guessers have guessed themselves into the inane conviction that the parallelism between the increase in the use of chemicals in foods and the increase in the incidence of cancer and other degenerative diseases constitutes damning evidence of a cause and effect relation between these phenomena. More justifiable than this would be the claim that the use of additives has contributed to the increase in our life expectancy—two equally parallel phenomena. Investigators have recently shown[7] that exercise in rats markedly reduces the rate of tumor growth compared with that in sedentary rats, and that extracts of exercised rat muscle, when given as an injection to sedentary rats, reduce tumor growth. So, if we look in the area of parallel and associated phenomena in search for our causes and effects, perhaps we could look with more favor on the increase in sedentary living in recent decades as the cause of that cancerous degeneration of our present generation which is claimed to exist by some dimly illuminated thinkers.

I would now like to indicate briefly some of the main problems faced by the investigator in examining additives and residues for their safety in man, and perhaps suggest some paths which might be followed effectively in attacking these problems.

EFFECTS ON ANIMALS VERSUS EFFECTS ON MAN

When the pharmacologist or toxicologist studies the responses of experimental laboratory animals to a chemical he never forgets the uncertainties involved in using his observations to formulate any prediction of the responses of the human being. Species differences in responses to drugs are commonplace, and these differences may be qualitative as well as quantitative. In fact, there are no two species of animals which react the same to all chemicals, although we frequently find two or more species which show very much the same kind and degree of reactivity to a given chemical. The greater the number of different species exhibiting the same reactivity, the greater is our faith that man will show the same responses. Man, however, may be the one species that differs from the rest, so that we can never rely

without reservation on any number of different species of laboratory animals to tell us the nature and intensity of the effects of a chemical in the human being. The ultimate definitive experiment, therefore, is on man himself. Few food additives have been tested in this way in well controlled experiments, although unfortunately, by virture of their presence in foods, may have been and are now being "tested" by the uncontrolled indiscriminate mass consumption technic from which little or no reliable information can be obtained. Desirable as it may be to conduct well designed experiments with food additives on man himself, the procedure has its limitations. Tests on man may more often be aimed cautiously at confirming the failure of a given dietary concentration to produce any effect, rather than searching for the minimal concentration which will produce an effect. This would be especially true if the animal experiments had shown any irreversible or slowly reversible reactions to the chemical, such as tumors, or pathologic changes in the kidneys or liver. On the other hand, some readily reversible effects have been regarded as safe to produce in experimental work with man.

For example, the artificial sweetening agent (Sucaryl®) has been tested in man given as much as 12 gm. a day for several weeks.[8,9] Prior studies on animals had shown this material to be mostly excreted in the feces, and the absorbed fraction to be rapidly excreted in the urine, with no evidence of accumulation in the body, conditions favoring a judgment of safety. In human subjects only a mild laxative effect was noted, which was found referable to an osmotic water-holding action of the agent in the intestine. In the absence of any evident action of Sucaryl on body tissues in amounts likely to be consumed, it is considered safe at essentially a 1:1 ratio between permissible intake and the amount which produces a minimal effect. This unusual situation is thought justifiable because here the minimal effect is considered to be a very unimportant effect, i.e., one not reflecting injury.

In a much bolder well controlled experiment a group of fifty-one men were fed DDT for eighteen months at a rate up to 200 times the normal dietary intake.[10] This produced no observable subjective or objective effects. Fat biopsy material taken from these men showed a concentration up to 20 p.p.m. in this tissue. In the range of intake involved in this study an equilibrium was reached in about one year, after which no further DDT concentrated in the fat. In comparison with other agents, DDT is recognized as one which is very strongly retained and concentrated in a specific tissue in the body. How much longer than eighteen months can a man tolerate 20 p.p.m. of DDT in his fat? Herein lies one of the limitations of experimentation on man. The feasible duration of the experiment is a relatively short segment of his total life span.

There is a detectable trend, however, toward experimental studies on the human subject. Controlled studies with some of the highly poisonous organic phosphate insecticides have been going on for some time.[11] Most of the investigations in man have been carried out on prison inmates, which would constitute a large reservoir of experimental material in this country. I see no real basic reason why chemicals cannot be tested even for carcinogenic effect on the skin of such subjects, since early skin cancer can be effectively treated.

In any case, however, it is evident that prior animal tests are required to provide enough background data to enable the investigator to proceed with safety in studies on human beings. Such studies are therefore not a substitute for, but rather an extension of, the experiments on laboratory animals. Their great value lies in the fact that, except for the length of the test periods, all conditions of the proposed use of the chemical additive can be exactly simulated in the experiment.

DISPOSITION OF THE CHEMICAL IN THE BODY

A method of study which has not been adequately exploited is the matter of what the body itself does with the additive, assuming that the substance has entered the body in the same chemical form in which it entered the food. Here, of course, a critical requirement is a good analytical method to enable one to follow the material in the blood, urine, feces,

organs and tissues. The investigator thus studies the fate or disposition of a substance. His findings can be of considerable value in deciding whether the substance has injurious effects in the body. For example, if it is not absorbed from the gastrointestinal tract and is excreted in the feces, then obviously it cannot become involved directly in any systemic physiologic functions. In this event, however, attention would still have to be given to the possibility of effects on the intestine itself or on the digestive processes, although past experience leads us to regard such effects, when they occur, as generally less serious than those which might result when the chemical is absorbed into the bloodstream. If it is found that the substance is thus absorbed, there are still conditions which would tend to minimize suspicion of harm. If the material is rapidly excreted in the urine, it is less suspect than if it accumulates in the body. If it is uniformly distributed throughout the body, it is less suspect than if it is selectively deposited in a specific vital organ where it could more readily reach a toxic concentration. If it is excreted in a chemically unchanged form, it is less suspect than if it reacts with body constituents and undergoes chemical modification.

One of the erroneous impressions given by the uninformed writers in the lay press is that all of these so-called "poisons" in our food continue to accumulate in the body as long as they are consumed. Contrary to this, it is much more common for a foreign chemical entering the body to be quite promptly eliminated. Furthermore, a general principle in pharmacology is that drugs are eliminated at a rate which is dependent on the amount in the body. In chronic toxicity work with substances which tend to accumulate a constant intake will produce a slow increase in the amount in the body until the rate of elimination equals the rate of intake. At this point of equilibrium the accumulation ceases and the amount present in the body will depend on the rate of intake. The faster the intake, of course, the higher will be the level at which an equilibrium is established.

To be sure, the results of disposition studies will not by themselves support any verdict as to the safety or hazard of a chemical additive. Their principal value would be to provide the investigator with an insight into the substance which should enable him to streamline and bring into better focus the time-consuming toxicity tests, and the laborious comprehensive pathologic studies which are too often carried out with no prior knowledge whatever of what parts of the body the chemical comes in contact with. The findings in such studies can aid greatly in the interpretation of symptoms observed and pathologic changes seen. They should indicate the direction in which attention should be given in subsequent studies of specific physiologic or biochemical systems of the body. Given an adequate analytical method, such investigations can frequently be accomplished on relatively few animals and in a relatively short time.

PHARMACOLOGIC INTERACTIONS BETWEEN CHEMICALS

One of the most challenging and difficult problems faced by the toxicologists in additive or residue work is the possibility that two or more foreign chemicals simultaneously entering the body may elicit reactions which are unexpected on the basis of knowledge of the effects of each compound separately. Many cases are known in which the presence in the body of one drug or poison, for example, intensifies, reduces or abolishes the action of another. Obviously it is the intensification, or potentiation, phenomenon which would concern us in the additive and residue field. Several pairs of the organic phosphate insecticides have been found to be many times more toxic when administered together than would be expected from their separate toxicities.[12–14] It seems almost certain that among the hundreds of chemicals that are added to foods there are other pairs of substances, which, if administered together in experimental animals, would produce a stronger reaction than anticipated from results derived from studies of each compound separately. It is probably just as likely, however, that pairs also exist which would produce a weaker reaction than expected or actually antagonize each other.

This whole problem, of course, is complicated

by the fact that it is not a matter of simple pairs of compounds but one of endless possible combinations of many. It is apparent that any attempt to test all combinations systematically would be highly impractical. A more rational approach is through a knowledge of the mechanisms responsible for the biological actions of the chemicals and a knowledge of their fate in the body. Information of this nature can lead us to suspect a possible insidious biological collusion between two or more chemicals. One chemical may have properties which could modify the absorption or distribution of another and in so doing increase the intensity of its action. Two chemicals which have the same mechanism of action may collaborate in such a way as to intensify their joint effect. One chemical may inhibit an enzyme system which normally functions to detoxify another, thus allowing the latter to accumulate in higher concentration or persist longer in the body. In the drug field, instances of potentiation or of antagonism between chemicals are common, and many of these have been discovered by putting to use a knowledge of basic pharmacologic mechanisms and body disposition. Similar instances among food additives are rare, partly because they have not been widely looked for, and partly because our information relating to mechanisms and disposition is inadequate. The accumulation of more information of this type would be highly useful if we are to develop a knowledge of the toxicologic interactions between chemical additives.

CHEMICAL STRUCTURE VERSUS PHARMACOLOGIC ACTION

It would be of tremendous advantage to all concerned with food additives and residues if the investigator could predict accurately the toxicologic and pharmacologic characteristics of a chemical substance on the basis of the already known characteristics of other substances with closely related chemical structure or similar physical properties. In work with drugs much valuable information has accumulated involving the relation between chemical structure and biological action. A number of homologous series of compounds have been found, for example, in each of which it is possible to interpolate or extrapolate some quantitative aspect of biological action and thus predict both the nature and intensity of effect on a member of the series which has not yet been tested. Knowledge and experience in this field, however has not reached the point where we can justifiably proceed with the practical use of a drug purely on the basis of such predictions. Each prediction has to be tested experimentally. There are too many series of compounds which do not favor us with a neat quantitative progression of a given action. Too many chemical series even show a change in the qualitative nature of the action as one goes from member to member, and a sudden major difference in intensity of action sometimes appears between two adjacent members of the series. For these reasons the pharmacologist still finds it necessary to test separately every single compound proposed as a food additive or pesticide in order to be certain of the nature and intensity of its various actions.

The study of structure-action relationships, however, not only in homologous series of compounds, but also in groups of compounds having a constant central group and differing drastically otherwise, have been and promise increasingly in the future to be highly fruitful. In the food additive field, perhaps the chemist can modify his molecules and provide the pharmacologist with products having at the same time minimum biological potency and maximum food processing potency. The pharmacologist with experience in the investigation of structure-action relationships can frequently suggest, on the basis of his observations, what molecular modifications might promise to minimize the undesirable effects he has encountered.

Some consideration should also be given to the physicochemical properties of chemical substances and how they might influence biological action. We know, for example, that lipid solubility and a small molecular size of a chemical tend to favor its transport across membrane barriers in the body, and promote its penetration into cells where it can exert its actions. Substances in the nonionized state generally penetrate all types of membranes

more readily than those in the ionized state. Lipid-soluble materials, which are not also water-soluble, gravitate toward the lipoid structures of the body such as the fat and central nervous system, while those compounds soluble in both water and lipid are more uniformly distributed, especially if they are in the nonionized state and their molecules are not excessively large. Thus, some chemical and physical properties which are easily measured by the chemist, can influence the absorption, distribution and excretion of chemicals and in this way affect their action in the body.

Other properties may not necessarily be related to biological action. For example, a property of one of the important groups of food additives, the emulsifiers or surfactants, is the ability to lower surface tension. Several years ago, the question arose whether this property of surface activity, *per se*, was a biologically undesirable feature of any food additive, or whether it would be possible to evaluate the safety of surfactants as a group on the basis of their surface tension lowering activity. The Food Protection Committee of the National Research Council considered this question with the intention of attempting an evaluation of the safety of surface activity as a property, and avoiding evaluation of specific substances possessing this property. This group's review of an extensive literature dealing with the biological effects of surface active agents resulted in the conclusion[15] that there are no toxic effects common to all surfactants, and that surface activity itself cannot be used as a measure of toxicity or of any definite biological effect in the whole animal. It was seen that different surfactants produce a wide variety of toxic effects, the latter apparently being more dependent upon chemical structure and other properties than upon the ability of the substance to lower surface tension. Although many physiologic and biochemical processes in the body are known to be dependent upon surface activity phenomena, we do not have any knowledge of a relation of this property of a chemical to its potential hazard as a food additive. It is, therefore, still found necessary to apply the classic biological criteria to each surfactant separately to determine its suitability for use in foods.

DOSE-EFFECT RELATIONSHIP

In any complete toxicologic work-up of a chemical compound the investigator studies the quantitative relation between dose and effect. This means that he measures the effect of each of a series of increasing doses in order to represent this relationship graphically. A study of this quantitative relationship can be of value in assessing the potential hazard of a chemical as a food additive. If the dose which produces a barely detectable effect is one hundredth of the dose which produces a serious or lethal effect, then clearly we are on safer grounds than if the minimal effect dose is only one-tenth that which produces a serious effect. Thus, a slow increase in the intensity of response to a chemical accompanying a relatively rapid increase in the intake level will have the effect of extending the range of safety substantially in addition to that imposed by the legal limit to which the chemical may appear in food. Accordingly, the slope of the dose-response curve might also be of use in determining the maximum concentration to be allowed in food in relation to the minimal effective concentration. Since any chemical can elicit several different biological responses the pharmacologist, in his study of a dose-effect relation, selects a response which has a practical bearing on the question of safety. Obviously, a response involving growth rate or mortality in relation to concentration in chronic feeding tests would be of much greater significance than would the blood pressure response in relation to the intravenous injection of single doses of the substance.

Our biggest problem in considering the dose-response relationship is the question of where is the lower termination of the curve. In other words, is there an amount of a substance, toxic in a given higher quantity, which is absolutely without effect on the body? This question is most worrisome in connection with materials which have been found carcinogenic in animals and with substances which tend to accumulate in the body. In the case of many additives and residues, however, the existence

of a lower termination of the dose-response curve has been accepted. Even for carcinogens the best qualified experts consider it reasonable to infer "that a no-effect level exists for man as well as for the laboratory animal."[2]

The future will probably show that no additive or potential residue needs to have a zero tolerance imposed upon it. Theoretically, there should be a finite quantity of anything which is harmless. In many cases, however, it will tax our ingenuity to invent methods for gathering data that can be used with confidence in estimating the maximum amount of a substance that can safely be present in the diet.

If we accept as fact that there is a harmless finite amount of any one substance, a sticky question still arises. Is there a harmless finite amount of each of many chemicals *simultaneously* entering the body? This question is forced upon us by the fact that our diet for the rest of our lives may contain hundreds of different chemicals. We have already intimated that there is no justification, in our present knowledge, for assuming the existence of any more additive action than antagonistic action among these many materials. But this is a wide open field for investigation.

INTERPRETATION OF EFFECTS

Investigators studying food additives and residues have thus far considered it desirable to regard any observed biological effect as a harmful effect until it can be proved not to be harmful. In short- or long-term toxicity studies, changes may occasionally appear which in themselves are not clearly detrimental to the general health and well-being of the animals as reflected in their appearance, appetite, growth, reproduction, various functional tests or length of life. In such cases difficulties may be encountered in proving the change to be either a sign of injury or of no importance to the health of the animal. The assumption that the change is a sign of injury until proved otherwise is based on the suspicion that our methods of observation are not adequate or sensitive enough to establish the change as a sign of injury, and that the observed effect is, in fact, a forerunner of injury which will later become evident.

An illustration of this problem is the moderate increase in the size and weight of the liver which is induced in rats fed certain classes of chemical substances. This larger liver appears entirely normal under the microscope, responds normally to liver function tests, and is accompanied by no detectable abnormalities of appearance, growth or length of life of the animal. The investigator may wonder in passing whether this is just a bigger and better liver and thus actually beneficial to the animal. But he is more likely to regard the change as due to an attempt by the body to compensate for some undesirable effect which remains undetected. He will suspect that his methods are inadequate and he will try to learn why the liver becomes larger or demonstrate by new methods whether the changes constitute an impairment in the health of the animal.

Another change which certain chemicals can induce in the animal throughout its life span, with no evidence of adverse effect on the over-all body economy, is a substantial reduction of cholinesterase activity in the blood. This enzyme is highly important in a number of physiologic functions, but at least some of it appears to be dispensable, probably because of the presence of an ample reserve supply. The question whether to regard a reduction of blood cholinesterase as a deleterious effect is somewhat different from the liver problem just described, although in either case animals retain good health. Knowing the importance of this enzyme and being aware that inroads are being made on the reserve supply it seems more justifiable to classify this effect as one approaching injury. There may well come a time when the reserve supply is needed.

This serves to illustrate some of the problems we face in dealing with food additives. Too often an effect observed is of a vague, indefinite borderline character, difficult to interpret and not always attributable without question to the substance under investigation. This situation is due to the fact that many chemicals added in the processing of foods are notably relatively low in toxicity, and in consequence of this, much of the animal testing is carried out with dose levels which produce either no effect or effects which are uncertain and in-

consistent. The ideal thing to establish with respect to the safety of an additive is its maximum no-effect level in long-term feeding tests, or what amounts to practically the same thing, the minimal effect level under the same conditions. Sometimes the minimal effect is quite elusive. It may disappear completely under slight changes in experimental conditions of which the investigator is unaware or over which he has no control—a different year, a different season of the year, a moderate change in the potency of the chemical under study, a change in the source of the animals, a change in quality or some other aspect of animal care, a minor change in the diet—one or a combination of these factors might be responsible. Experimental conditions in different laboratories are never exactly the same and may account for the variable observation of different groups of investigators, especially in the realm of the borderline minimal effects.

NEED FOR NEW DEVELOPMENTS

Obviously, we have problems on our hands. These problems remain with us because the too few scientists who are working in this field have been kept more than busy trying to grind out their safety evaluation programs on too many chemicals. They have had little opportunity for the basic research needed in order to develop new ideas and new methods to facilitate food additive evaluation procedures. We need new and more sensitive biological criteria of chemical injury which are more well defined and less susceptible to variable interpretation or misinterpretation.

There is much room for progress in what to look for, how to look for it, and how to interpret what is seen. Progress will be slow in the present shortage of trained investigators and the shortage of interest in those aspects of additive and residue toxicology where attention is needed most, i.e., in the study of the chronic biological effects of chemicals with relatively low toxicity and slow rate of onset of action. The pharmacologist has always displayed much more interest in the highly poisonous pesticidal chemicals simply because of the more dramatic character of their actions and potential effects. But there is sufficient challenge in the problems involving the toxicologically less spectacular chemicals to attract the interest of scientists who become aware of these problems.

In addition to the need for more pharmacologists or toxicologists we can use to great advantage the talents, tools and training of the biochemist, physiologist, cytochemist, experimental anatomist and pathologist, and other specialists. The application of diverse scientific talents is needed to bring new light to this field.

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Panel Discussion of Additives and Residues in Human Foods

Moderator: ROBERT L. JACKSON, M.D., *Columbia, Missouri*
H. R. BIRD, M.D., *Madison Wisconsin* PAUL L. DAY, M.D., *Washington, D. C.*
JOHN F. MAHONEY, M.D., *Rahway, New Jersey* JULIUS M. COON, M.D., *Philadelphia, Pennsylvania*
ROBERT N. GOODMAN, *Columbia, Missouri*

DR. JACKSON: The purpose of this symposium is to review the current status of knowledge in this important field, to help the educated guessers with the difficult task assigned to them, and to stress the importance of the subject to students of nutrition as well as to others interested or involved.

The Food and Nutrition Council of the American Medical Association and the Food Additives Committee of the Food and Nutrition Board of the National Research Council began to sense the serious potential difficulties of solving this problem and began to construct effective methods of controlling additives long before the public concern about the cranberry and stilbesterol episodes.

Dr. Bird, will you please begin the discussions?

DR. BIRD: There are two questions here regarding the enforcement of the time limit between the feeding of the additive and the slaughter of the animal. I would make one correction in the question which states "How can the time limit be policed? Example: five days before killing chickens to stop antibiotics." This rule does not apply to antibiotics as they can be fed right up to the time of slaughter and still leave no residue. This time limit does apply to arsenicals and to the feeding of stilbesterol to beef cattle, dienesterol diacetate to chickens. It also applies to some of the miscellaneous group of additives and some of the coccidiostats. Nobody is going to go out to the broiler houses to make sure that the chickens are changed over to a different feed three or five days before they go to market. Undoubtedly there are instances, perhaps quite a few instances, in which this regulation is ignored. I would say that even if it is ignored, there is really no hazard involved. The figures for arsenicals, for example, show that if they are fed right up to the time of slaughter, the residue will be a fraction of a part per million. If the time limit is adhered to, it will be a mere trace—hardly detectable. Even the amount that would be left after feeding right up to the time of slaughter is still probably less than half or less than a third of the residue permitted on sprayed fruits. The time limit was included as an extra safety precaution, and obviously it should be adhered to. This requires a continuous program of education.

With respect to antibiotic residues in milk, it is possible to determine whether or not regulations are being followed by assay of the milk, because there should be no antibiotic in milk as it goes to market. There have been prosecutions on this point. It is easier to catch such lots of milk as they go into dairy manufacture than it is to catch them as they go into market milk. Milk moves quickly from the producer to the consumer. Market milk, however, requires more steps for processing so that there is more time to pick these things up. This also requires not only punitive measures, but also a continuing program of education.

QUESTION: "Why do you put the safe requirement for an additive in third place? It seems to me that this should be in first place."

DR. MAHONEY: I think the question refers to the section indicating some of the problems we face in justifying a food additive research

program. I indicated that first we must develop an additive which is effective; second, one which in the terms of taste, flavor and cost will be practical for food use; and third, one which must be safe. They are in this order because this is how I would develop a new food additive. If I were concerned with developing leads to families of new compounds in which one member might be tailormade to be satisfactory as far as safety is concerned, I would first attempt to find an effective compound, without regard to whether it were safe or not. Having developed an effective compound (or perhaps several effective compounds), I would then be concerned with determining in the quickest and easiest way possible whether or not these were likely candidates for further consideration. The easiest way to accomplish this is on the basis of such things as cost, color, flavor and taste, and whether or not they would be practical for food use. Only then, would I authorize a testing program to determine whether or not the additive which was effective and otherwise practical for food use was also safe. The reason for this is that the safety testing program ordinarily runs over a period of two to two and a half years. It may cost as much as $2,000,000 and is not something to be entered into lightly. One would not want to carry three or four compounds through such a testing program if, by other means, it were possible to limit the choice to one. By no means did I mean to indicate that safety is in third place as far as a criteria of food use is concerned. There is no qualification to any of these requirements. A compound must be safe, it must be effective and it must be practical for food use without qualification.

QUESTION: "Would one of the speakers like to comment upon mercurials and other "dangerous substances" which are present in lipsticks? Small amounts of lipstick are ingested each time the lady eats and, over a period of years, the dangerous components are undoubtedly harmful. Are there any safe lipsticks on the market?"

DR. DAY: To my knowledge there are no heavy metals, such as mercury, used in lipsticks today. Although many substances are undoubtedly used in lipsticks, they are composed essentially of a wax (such as beeswax, a vegetable oil) castor oil, lanolin, some mineral oil or petrolatum, and a certified Food, Drug and Cosmetic color. Unfortunately, a number of the so-called coal tar colors most favored for lipstick have recently been taken off the certifiable list by the Food and Drug Administration because they are not, in fact, harmless. This action was necessary because of the specific wording of the 1938 Food, Drug and Cosmetic Law, which still governs the use of coal tar colors. At the time that law was passed it was believed that coal tar colors which had been used for many years were completely harmless. Only after a number of people became ill from eating candy which contained an inordinate amount of a food color were studies started on the safety of the coal tar colors. Toxicologic feeding tests, using rats and dogs, have shown that many of the certifiable colors are not safe. A color which kills rats, or depresses their growth, or which makes them anemic is not "harmless." In many of these toxicologic tests the amount of color fed is much greater than any person could possibly ingest, but the law says "harmless." No person I know seriously believes that there is danger of a woman getting enough color from lipstick to cause her harm, but that is not the point. Even though the law is somewhat unrealistic, in view of our present knowledge, a Federal agency cannot flout the law as long as it is on the books.

The Congress is now considering legislation which would permit the setting of tolerances for food, drug and cosmetic colors, taking into consideration the amount of use in relation to their toxicity. If this law is passed by the Congress, many of the colors now banned may be permitted for certain uses.

QUESTION: "Why are cigarettes—which are considered by some to be carcinogenic—not included with food additives if the purpose of the Delaney Amendment is to protect the population from ingestion of carcinogenic compounds?"

DR. DAY: The Food, Drug and Cosmetic Law covers foods, drugs and cosmetics. Cigarettes are not food; although they contain nicotine they are not classed as drugs and no

cigarette manufacturer would want his product considered a drug; and they are not cosmetics. Last year a brand of cigarettes was placed on the market which, by its name and advertising, implied a usefulness in weight reduction. Shipments were seized by the Food and Drug Administration and the product was charged with being a new drug for which proper clearance had not been obtained from the Food and Drug Administration. In the trial the Court found that the cigarette was a new drug within the meaning of the law, and since a New Drug Application had not been made effective by the Food and Drug Administration the court ordered the shipments destroyed.

A similar question to this was put to Secretary of Health, Education and Welfare, Arthur S. Flemming, at a television college news conference a short time ago. One of the student editors asked why, since he had banned the sale of cranberries which contained a cancer-producing chemical, he did not also ban the sale of cigarettes. Dr. Flemming pointed out that there had been a great deal of publicity given the relation between cigarette smoking and lung cancer; that he, himself, had publicized the Public Health Service viewpoint that smoking is probably a cause of lung cancer. Furthermore, one does not have to smoke cigarettes. If a person, knowing the danger, chooses to smoke, that is his privilege as a free member of society. On the other hand one must eat; the government has a responsibility to see that cancer-producing chemicals do not get into the public's food.

QUESTION: "What is the panel's opinion of William Longgood's book "The Poisons in Your Food?"

DR. COON: This was the book to which I referred earlier. A comment with the question is that the author has a B.S. degree in journalism from the University of Missouri. A further comment is that Longgood questions the ability of the doctor in the DDT experiment. This refers to a group of investigators at a U. S. Public Health Service laboratory in Savannah, Georgia, who published the results of their studies on man in the *Journal of the American Medical Association* about four years ago. I have never heard of any adverse comment concerning the ability of this group. Their work, of course, was performed on human beings, and investigators are beset by many difficulties in a field of this kind when working with human beings. For example, the type of data that is obtainable from animals is frequently not obtainable from human beings. In my opinion Longgood's criticism is wholly unjustified.

Before calling for the panelists' opinion of Longgood's book, I would like to quote from a review of the book written by Dr. William Darby, who is the chairman of the Food Protection Committee, about which you have heard this afternoon. This review appeared in *Science* about three weeks ago. I quote: "The muckraking in this book employs all the well-known methods of the irresponsible purveyor of the sensational." In another place, Dr. Darby says: "Longgood's book will readily be recognized for what it is, by any scientist with so little to do that he takes time to read it." I might add that Longgood apparently received good training in journalism here at the University of Missouri but he took tremendous liberties in applying his training to a scientific subject.

QUESTION: "Since there is more glory in the development of a useful additive than in checking its tolerance and toxicity, how do you propose to increase the number of qualified people in the lab?"

DR. COON: In the first place, one must say that checking the tolerance and toxicity is a very important part of the development of a useful additive. However, to answer what was meant here, I believe that the main way to increase the number of qualified poeple in this field is to stimulate interest. The purpose of this symposium, I am sure, is to stimulate interest as well as to inform. We had a full-day symposium at the Federation meetings in Chicago in which this same matter was discussed along with the toxicologic problems that exist in areas other than food additives and residues, including contamination of the atmosphere, contamination of water, radioactive materials, drugs and cosmetics. Stimulation of interest is the primary issue here. I might add that the National Institutes of

Health, through its Toxicology Study Section, is actively programming and trying to stimulate the interest of people to do research on these toxicologic problems. This is a new Study Section, so I am glad to have this opportunity of doing a little programming for it and to indicate to those who have some incipient interest in these matters to apply for support for their research.

QUESTION: "Although there might not be a significant amount of residue from an additive present, is it not possible that this additive could have caused the production of some toxic substance that is present, but for which no tests have been made?"

DR. BIRD: I would point out that in nearly all cases when a toxic material is given to an animal, the animal detoxifies, or partially detoxifies, the compound and excretes it or the metabolites. There are very few known instances in which a toxic compound is converted by the animal into something even more toxic. I would also point out that in the case of the estrogens and the arsenic compounds, which are the two groups of greatest interest in animal production, the tests that are made are actually tests for total activity and not for any one compound. That is to say, although I probably spoke about testing for diethylstilbestrol that is really not what is performed. The test is for total estrogen in the tissue. We are alert to the possibility that in any given case a metabolite might be left as a residue. I think I mentioned that in the case of nicarbazin they do find dinitrocarbanilide. As as matter of fact, that is the residue of interest in that case. I do not know whether there would be anything to say in connection with plants or foods of plant origin in this connection.

DR. GOODMAN: There is, of course, always a possibility that breakdown products may be formed in the plant that are more toxic than the original substance. I do not know of any specific instances in which a toxic breakdown product has been picked up in plants. It has been reported that the antifungal antibiotic, cycloheximide, when applied in the acetate form to the plant, is converted to the more active form once it is in the plant. It is also possible that modifications of the original substance may occur which are so subtle that differentiation between the metabolite and the parent molecule are beyond our means of detection.

DR. DAY: If I understand the question read by Dr. Bird correctly, I know of one example, although it involves an oxidation product of a pesticide rather than a metabolic product. This is the pesticide heptachlor—a chlorinated hydrocarbon. Its use on feed crops was approved with the provision for "no residue" in milk. The data available at the time the petition was approved by the Food and Drug Administration showed that its use on feed and forage would leave no residue in milk. It was later learned, however, that an oxidation product of heptachlor—an epoxide—does appear in milk after use of the pesticide on feed crops. Since the epoxide is just as toxic as heptachlor, the use of heptachlor has now been restricted.

QUESTION: "Why did Secretary Flemming use the method of a press conference and a news release to announce the Food and Drug Administration's plan regarding cranberries, and inform the public rather than simply enforce the law?"

DR. DAY: I do not know the reasons for Dr. Flemming's decision to handle the matter in this way, but I am confident that he believed there was imminent danger to the public which could not be handled by the usual methods of seizure and other legal means. There is some background information necessary to an understanding of the case and this question, which is actually three questions, brings it out. This reads: "What was the insecticide?"

The material used on cranberry bogs was not an insecticide; it was a weed-killer, chemically known as 3-aminotriazole. Dr. Goodman showed the structural formula for it on one of his slides. It is an effective and cheap weed-killer. Its acute toxicity is rather low; that is, quite a large single dose would be required to kill a person or even make him sick. But toxicologic studies have shown that it acts to suppress the action of the thyroid gland, causing an enlargement of the thyroid which later may become cancerous. Under

the Delaney Clause of the Food Additive Amendment its use must be prohibited when a residue would remain on a food crop.

Aminotriazole was used on cranberry bogs in Oregon and Washington in 1957 and as a result about 2.5 million pounds of the berries were contaminated. The substance had not then been registered with the Department of Agriculture. In January 1958 it was registered with the Agriculture Department for use on cranberry bogs under conditions in which residues would *not* remain on the fruit. The regulations specifically permitted its use *after* harvest. When used *before* harvest the chemical is taken up by the roots of the cranberry plant and some of it deposited in the berries. As a result of the contamination of about half the northwest state's crop, the part contaminated was segregated and placed in cold storage.

When it was learned in 1959 that toxicologic tests had shown aminotriazole to be a "cancer-producing substance," the Food and Drug Administration rejected a petition for a tolerance on cranberries. When in the fall of 1959, it was learned by the Food and Drug Administration that the herbicide was being used on cranberry bogs in Washington and Oregon contrary to regulations, chemical examination of cranberries for the chemical was started. When it was found that cranberries offered for sale in interstate commerce were contaminated, Secretary Flemming made his public announcement. It was patently impossible to collect samples and analyze every shipment of cranberries in the short time remaining before the Thanksgiving season.

The 2.5 million pounds of contaminated cranberries from the 1957 crop were destroyed, voluntarily, in the fall of 1959. I lived in the state of Washington for several years and always thought that Washingtonians were intelligent people. I am puzzled as to why, after ruining their 1957 crop, they repeated the mistake in 1959.

The final part of this question is: "How much did the cranberry packers lose?" The complete answer to that is probably unknown. It has been decided that the Federal government will reimburse growers for their *uncontaminated* cranberries, and it is rumored that the total will be about 8 million dollars. No doubt that is a small part of the total loss, for it will be many years before the cranberry-cancer episode is forgotten. I am told that they are referred to as "cancer berries" in some places.

QUESTION: "Even if the food additives are investigated as to their freedom from toxic effects, is it not conceivable that the addition of numerous additives obtained by the consumer from numerous sources may have an additive effect that may be toxic?"

DR. MAHONEY: I think it is unlikely that such an additive effect would be sufficiently great to be a safety problem. There are a number of limitations that act to prevent the accumulation of a harmful amount of additive from a variety of sources. First, as you may know, for an additive to be approved as safe for food use, it must have a considerable margin of safety; ordinarily, a hundred to one or more. Second, if the additive is one that requires a tolerance for safe use, the tolerance is limited to the minimum quantity of additive required to do the job. Third, the Food Additive Amendment makes provision for treating a series of related compounds, where additive toxicity might be anticipated, as a class; and establishing tolerances for the class and not for individual members of the class. Fourth, in establishing tolerances, consideration is taken of the probable use of the additive in a variety of foods. One of the problems of concern to industry is how to establish an equitable basis for tolerances in specific foods in which the additive is one with a variety of applications.

QUESTION: Why is increased fat considered a sign of good growth in poultry when increased protein is what is desirable for nutritional purposes?

DR. BIRD: I realize that fat is sort of an obscene word now, and one hestitates to mention it in a mixed group; however, this being a medical school audience, I assume that we can talk about it freely. The fact is that chicken meat and turkey meat are much lower in fat than any other meat which is commonly consumed. This is the point I want to make.

If you want to eat a low-fat diet, and still eat meat; well, eat more chicken and turkey. If you have ever had chicken or turkey and thought it was dry or unpalatable, and this occasionally does happen even with this very excellent product, this probably was because it was too low in fat. This is the reason that attempts have been made, by using diethylstilbestrol or by feeding dienestrol diacetate, to bring about an increase in the fat content of poultry meat. We used to think that this could not be done by dietary means. We know now that it is possible to increase the fat content of poultry somewhat by proper selection of diet as well as by hormones. This is the reason why estrogen has been and is still being used in the form of dienestrol diacetate in a small proportion of the poultry produced.

DR. JACKSON: Dr. Bird, What do you think the government should do with all those caponettes they just took over? Is not the stilbesterol mostly in the liver and glandular organs? Could they be used?

DR. BIRD: It is mostly in the liver, skin and some of the other organs which are not commonly eaten. Yes, this material could be used. I would not hesitate to eat it myself, including the liver. I do not know what is going to be done with it.

QUESTION: "What is meant by zero tolerance?"

DR. COON: I used the term rather loosely without definition; actually, there is no exact meaning for zero tolerance. The highest level of an additive or residue that is permitted in food is termed the tolerance for that particular substance. A zero tolerance may be an amount that cannot be detected by any analytical methods available, or it may mean that there will be no permission to use the particular chemical in any stage of production of the food. Dr. Day may like to elaborate on this particular issue.

DR. DAY: I was listening, Dr. Coon, for you to define zero tolerance. I have been wondering about it for a long time. I do not understand what it means.

QUESTION: "Is the increase of liver size which is induced by certain substances most likely due to the development of enzymes to eliminate drugs?"

DR. COON: There is undoubtedly some biochemical process involved in the response to these agents that induced a moderate increase in liver size. There has been some searching for such a process, but it has not yet been located.

QUESTION: "Is there any scientific reason why a tolerance cannot be set for a chemical thought to be a carcinogen when a tolerance is being established on the level of radiation that might cause cancer?"

DR. DAY: This involves a fundamental and simple principle which seems to have eluded many intelligent people. There has been a good deal of loose and unscientific thinking on this subject, even by some good scientists, and this question points up the problem. The question just read might be paraphrased: "If you prohibit the addition of arsenic to food, why not prohibit the sale of shellfish, because there may be far more arsenic in shrimp or lobster than one would get from eating chicken which had been fed a feed containing an arsenical drug?" This is a hard question to answer, but perhaps I can answer it this way: If there is any danger of arsenic in food causing cancer, then why subject ourselves to any more arsenic than necessary? There is no way of keeping arsenic out of shellfish, but we can keep it out of chicken.

There is no way of preventing the contamination of vegetables and milk with strontium-90 because of the fallout from bomb testing. But the Food and Drug Administration has a legal mandate to prevent any *additional* radioactivity from being added to food. You all know something about the enormous project which has been carried out by the Department of Defense and the Atomic Energy Commission on the sterilization of food by radiation. Hundreds of thousands, in fact millions, of dollars have been put into this study. But the project has run into trouble because it has been found that to effectively sterilize some foods it is necessary to use such intense radiation that radioactivity is induced in the food itself. This clearly brings it under the interdiction of the anticancer clause of the Food Additive

Law; the so-called Delaney Clause specifically mentions radioactivity. Here again we find an apparent contradiction similar to the case of arsenic in shellfish as compared to arsenic in chicken from arsenical drugs. All natural foods contain radioactivity—potassium-40 and a small amount of carbon-14. Also, we are continuously subjected to cosmic rays and to a small amount of radiation from the rocks of the earth's crust. Natural radioisotopes in food and soil, and cosmic rays cannot be prevented. But we *can* prevent the addition of any further radioisotopes in food, and we are required to by the Food Additive Law.

QUESTION: "Is there any justification of any trade secrets in terms of public welfare? Put in other words, is it unreasonable for people to ask what am I eating and how is it prepared?

DR. MAHONEY: In my talk I mentioned the disclosure of trade secrets as one of the immediate problems being faced by industry under the Food Additives Amendment. I had specific reference to the food flavor formulator who may draw from several hundred different compounds in producing his various imitation flavors. His stock in trade lies in his ability to combine these products skillfully to give a close imitation of a natural product. These combinations are not patented. If their compositions were disclosed, they would more readily be copied by a competitor.

I do not believe it is necessarily in the public interest that the details of such products be publicly disclosed. I am not sure it would benefit the consumer to have on a food package a list of fifteen, twenty or twenty-five different chemicals with long names as evidence that the food was artificially flavored. I am inclined to believe that the present practice of simply indicating that the food is artificially flavored is sufficient. This has proved satisfactory through the years.

DR. GOODMAN: I would like to comment on one of the points brought up by Dr. Coon, namely the use of human volunteer subjects. I believe that researchers could investigate the possibility of obtaining human volunteer subjects at State Penal Institutions, and hospitals. In this respect I should like to state that we are deeply appreciative of the assistance rendered by both the inmates and staff of the Missouri State penitentiary at Jefferson City.

DR. JACKSON: Representing the faculty of the School of Medicine of the University of Missouri, I wish to thank everyone, particularly the speakers but also the responsive audience, for this excellent symposium.

Clinical Reports

Present Status of Vitamin B_6 Metabolism

DAVID BAIRD COURSIN, M.D.*

THE existence of vitamin B_6 as a dietary essential in "rat acrodynia" was first recognized by György[1] in 1934. The vitamin was subsequently isolated[2a] and its basic chemical structure identified by Keresztesy.[2b] That same year, Harris and Folkers[3] synthesized pyridoxine, providing adequate material for extensive research into its biochemistry and physiologic action in microorganisms, animals, and man.

BIOCHEMISTRY

Initially it was thought that vitamin B_6 was entirely pyridoxine. However, the elegant microbiologic studies carried out by Snell[4] and his co-workers revealed the existence of three separate interconvertible members of the vitamin B_6 group. These were shown to be pyridoxine (PIN), pyridoxal (PAL), and pyridoxamine (PAM). Figure 1 shows the chemical structure of these forms of the vitamin as well as their metabolic end product, 4-pyridoxic acid (PIC).[5] It has been demonstrated that the biologically active forms of vitamin B_6 were phosphorylated at the 5 position pyridoxine phosphate (PIN PO_4), pyridoxal phosphate (PAL PO_4), and pyridoxamine phosphate (PAM PO_4).[6,7] Of these forms, pyridoxal-5-phosphate has been shown to be the active coenzyme that catalyzes a large number of biochemical reactions in the metabolism of amino acids, fats, and carbohydrates.[8]

The following list of some of the more important reactions that require pyridoxal phosphate is adapted from Meister.[9,10] Transamination is the only one of these that will also proceed with pyridoxamine phosphate as its coenzyme.

A. Amino Acid Metabolism

(1) Transamination: $RCHNH_2COOH + R^1COCOOH \rightleftarrows RCOCOOH + R^1CHNH_2COOH$ e.g., glutamate + oxalacetate → alpha ketoglutarate + aspartic acid.

These substances are important constituents of both the citric acid cycle and the urea cycle. The following sequence of reactions involved in this transamination brings out clearly how pyridoxal phosphate is involved in their metabolism.

glutamate + PAL ⇄ glutamate PAL
glutamate PAL ⇄ ketoglutarate PAM
ketoglutarate PAM + oxalacetate ⇄ ketoglutarate + oxalacetate PAM
oxalacetate PAM ⇄ aspartic acid PAL
aspartic acid PAL ⇄ aspartic acid + PAL

(2) Elimination and addition reactions:
e.g., Dehydrase: Serine → pyruvate
$HOCH_2CHNH_2COOH \rightarrow CH_3COCOOH + NH_3$
Desulfhydrase: Cysteine → pyruvate
$HSCH_2CHN_2COOH \xrightarrow{H_2O} H_2S + NH_3 + CH_3COCOOH$
Synthetase: Serine → tryptophan (Bacteria)
$HOCH_2CHNH_2COOH \xrightarrow{H_2O} (CH_2{=}CNH_2\text{-}COOH) \overset{\text{indole}}{=\!=}$

(3) Racemization:

$$\begin{array}{ccc} NH_2 & & H \\ R\text{—}C\text{—}COOH & \rightarrow & R\text{—}C\text{—}COOH \\ H & & NH_2 \end{array}$$

e.g., L-methionine → D-methionine (Bacteria)

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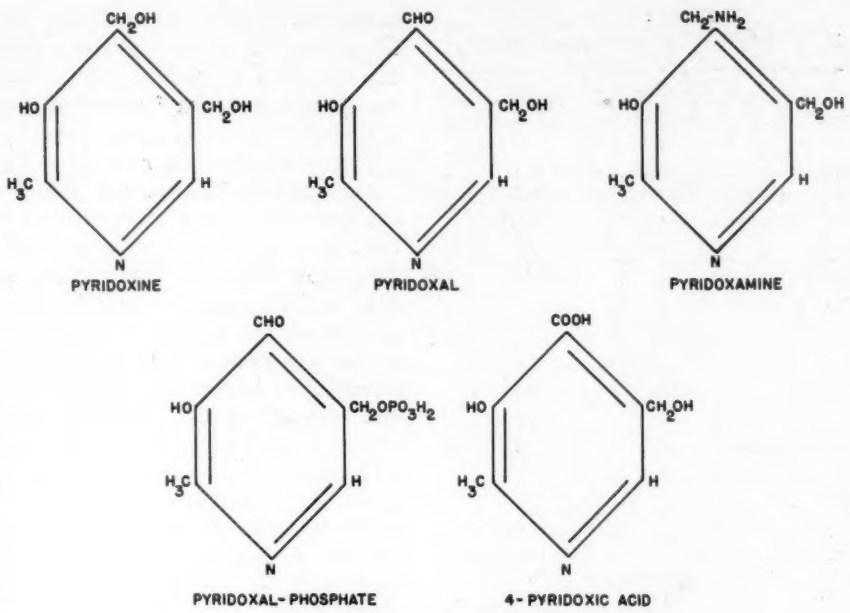


FIG. 1. The forms of vitamin B_6.

(4) Decarboxylation:
$RCHNH_2COOH \rightarrow RCH_2NH_2 + CO_2$
e.g., L-glutamate → gamma aminobutyrate + CO_2

(5) Amine oxidation:
$RCH_2CH_2 + (O) \rightarrow RCHO + NH_3$
e.g., Histamine + (O) → imidazoleacetaldehyde + NH_3

B. Carbohydrate Metabolism

Beaton[11,12] first called attention to the diminution of fasting blood sugar, pyruvic acid, lactic acid, and glycogen in rats receiving a vitamin B_2-deficient diet. He also demonstrated a decrease in the activity of the liver enzymes of lactic acid dehydrogenase, aldolase, and catalase.[13] These changes as well as alterations in fat metabolism occurred even earlier than those for amino acids.

More recently, Baranowski et al.[14] have reported the presence of pyridoxal phosphate in phosphorylase, the enzyme responsible for the breakdown of glycogen to glucose-1-phosphate. Both the *a* and *b* forms of phosphorylase have been shown to contain at least several moles of pyridoxal phosphate.[15]

C. Fat Metabolism

Vitamin B_6 deficiency in animals has repeatedly been shown to produce changes in the metabolism of fats and fatty acids, and to interfere with the conversion of protein to fat.[16–18] More recently, Mueller et al.[19] have demonstrated a fall in polyunsaturated fatty acids as a result of vitamin B_2 deficiency.

Each of the enzymes with which vitamin B_6 is active as a coenzyme has its own individual affinity for the vitamin, resulting in certain as yet undefined minimal requirements and optimal concentrations of vitamin B_6 which are necessary for its activity.[20–22] In some situations, excess concentrations of vitamin B_6 may possibly have an inhibitory effect upon a reaction.[23]

ASSAY METHODS

Since its discovery, a number of assay methods have been devised in an effort to measure the vitamin, as well as to evaluate its physiological effects.[24] Originally, the bioassay method using the "rat acrodynia" changes and later the effects on growth were used to determine the degree of vitamin B_6

TABLE I
Vitamin B_6 Content of Various Types of Foods[28]

Food	μg./gm.
Milk	
Cows*	1.1
Human*	0.15
Eggs	0.22
Meat	
Beef liver	7.3
Beef steak	2.8
Chicken	1.2
Pork, ham	3.9
Grain	
Wheat	5.8
Oats	0.92
Rice	3.4
Corn	4.9
Yeast, Brewer's, dry	47.3
Vegetables	
Beans, lima	6.0
Carrots	2.1
Onions	0.63
Potatoes, white	1.8
Soy beans	10.2
Fruits	
Apple	0.26
Bananas	3.2
Orange	0.31

* Reported as microgram of pyridoxine per gram or microliter.

deficiency.[25] With the recognition of three separate forms of the vitamin, microbiologic procedures were developed utilizing Saccharomyces Carlsbergensis for total vitamin B_6; Streptococcus faecalis for pyridoxal and pyridoxamine; and Lactobacillus casei for pyridoxal.[26,27] These methods have been widely used in establishing the standards for food content of the members of the vitamin B_6 group. Table I lists some of the values obtained for representatives of different foods.[28] It should be noted that in plant sources, vitamin B_6 is present primarily as pyridoxine, whereas animal sources contain mostly PAL and PAM.

These technics have been satisfactory, but pose certain technical difficulties which have stimulated a number of workers to develop biochemical technics for assaying vitamin B_6. Early efforts showed that if pure solutions could be obtained, spectrophotometric technics were applicable.[29] In biologic samples, however, there were a number of other substances which interfered with these procedures. More complex means of determination have therefore been devised, such as ion exchange,[30] the tyrosine decarboxylation studies on white blood cells,[31] the apotryptophanase reaction,[32] and the spectrophotofluorometric technic.[33]

Another test that has had wide usage and has provided a great deal of important information is the tryptophan load test.[34] In this procedure, the chemical sequence outlined in Figure 2 is utilized by loading the subject with a measured quantity of tryptophan and determining the xanthurenic acid excretion.[35] As can be seen in the diagram, a deficiency of vitamin B_6 changes the normal course of breakdown of tryptophan, forcing it to alternate pathways resulting in the production of excessive xanthurenic acid. This defect is corrected by the administration of vitamin B_6 resulting in a marked decrease in xanthurenic acid excretion.

The measurement of levels of serum glutamic oxalacetic transaminase is another technic that has been used to indicate the availability of vitamin B_6.[36,37] A decrease in the activity of this enzyme system has been interpreted as resulting from a decrease in the coenzyme.

STUDIES IN ANIMAL METABOLISM

Experimental studies in animals have shown vitamin B_6 to be a dietary essential in all species. Work with vitamin B_6 deficient diets routinely produces depletion of vitamin B_6 in the tissues with subsequent loss of appetite, increase in central nervous system irritability, decrease in growth, loss of weight, and death.

Hypovitaminosis may be created by the use of a diet containing suboptimal amounts of vitamin B_6, thereby affecting a number of normal metabolic pathways without causing death. Similar changes can be brought about by using vitamin B_6 anti-vitamins such as deoxypyridoxine, which is capable of replacing pyridoxal phosphate in its coenzyme position, thereby interfering with its normal reactions.[38] Another group of substances, such as isonicotinic acid hydrazide, can be administered. These combine with pyridoxal to form an inactive pyridoxal hydrazide compound.[39] This reduces the amount of pyridoxal available

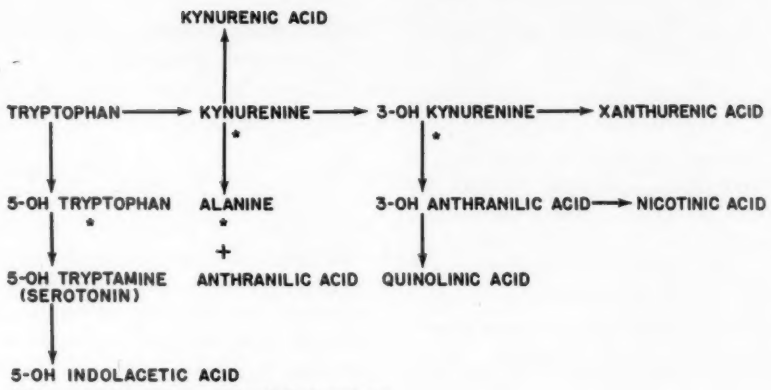


FIG. 2. The intermediary metabolism of tryptophan.

for enzyme activity, thereby producing a deficient state. By one or a combination of these technics, a number of individual enzyme systems have been explored, showing the importance of vitamin B_6 in the intact animal. All the phenomena produced by the deficiency of vitamin B_6 can be corrected by adding the vitamin to the diet and by stopping the administration of the vitamin inhibitor. The following are some of the observations made with these technics.

Skin

Definite changes have been noted in vitamin B_6 deficiency since the existence of the vitamin was first suspected by Goldberger and Lillie[40] in 1926. The "pellagra-like" lesions they described were subsequently shown by György[41] to be similar to the "rat acrodynia" changes. Similar skin changes have been demonstrated in almost all animal species except cattle.[42] Defects consist of erythema, scaling, hyperkeratosis, edema, and eventually alterations in sebaceous glands and hair follicles. Mueller and Vilter[43] have used vitamin B_6 deficient diets and deoxypyridoxine in monkeys and has produced similar changes.

Blood Forming Tissues

On a vitamin B_6-deficient diet or after the administration of deoxypyridoxine there is atrophy of lymph follicles with progressive decrease in circulating lymphocytes. This is accompanied by a general decrease in total white blood count and a relative increase in neutrophils.[44,45]

Erythropoietic tissue also appears to require vitamin B_6, probably for protoporphyrin synthesis.[46] In animals on deficient diets hypochromic, microcytic anemia develops and their capacity to regenerate normal erythrocytes is poor.[47] In many experimental studies the appearance of anemia has been one of the earliest signs of abnormality.

Endocrine

The adrenal glands in vitamin B_6-deficient rats have shown progressive enlargement of zona with encroachment on the zona fasciculata. This change produces a progressive loss of sudanophilic lipoids, birefringent material, cholesterol, and vitamin C content.[48] Steroidgenesis has also been shown to be altered in these animals.[49]

Vitamin B_6 deficiency affects pituitary and ovarian functions as evidenced by alterations in fertility and reproduction in rats.[50] There appears to be a normal production of gonadotrophic hormones, but with alterations in activity of the follicle-stimulating and interstitial cell-stimulating hormones.[51,52]

In mice, pyridoxine with vitamin B_{12} and folic acid was shown to prevent the formation of congenital anomalies that usually appeared

with large doses of cortisone administered during pregnancy.[53]

Central Nervous System

Nervous system changes in vitamin B_6 deficiency were first described in rats by Chick et al.[54] in 1950. Histologic studies have shown progressive demyelinization of nerve fibers, axis cylinder degeneration, and cellular atrophy in rats maintained on vitamin B_6-deficient diets.[55] Other species have shown similar symptoms of hyperirritability, grand mal type seizures and coma.[56,57] The animals may die or may recover with a persistent disability of repeated convulsive seizures. Deoxypyridoxine and isonicotinic hydrazide have been found to produce convulsive seizures apparently through the mechanism of interference with normal vitamin B_2 metabolism.[58,59]

Miscellaneous

Vitamin B_6 has been shown to be important in a number of other biologic systems. In animals antibody synthesis is impaired by a deficiency of vitamin B_6.[60] This mechanism further seems to be related to the problem of the successful taking of homografts in animals maintained on diets low in vitamin B_6.[61] The growth of sarcomas in mice has been found to diminish by reducing pyridoxal phosphate in the cancerous cells.[62] Vitamin B_6 deficiency in monkeys results in degenerative vascular changes with atheromas and atherosclerosis.[63] Prolonged deficiency of pyridoxine produces changes in normal tooth structure with a marked increase in caries.[64] Radiation exposure affects the normal metabolism of vitamin B_6 in the intact animal.[65]

This brief review of some of the more significant metabolic changes seen in animals with vitamin B_6 deficiency provides an insight into the extensive importance of this vitamin and also into the complexity of the problem of identifying the experimental role of vitamin B_6. While it would be erroneous to draw direct conclusions from animal work to human metabolism, there is much that can be derived from this experimentation that may provide a better understandng of clinical phenomena that have been seen in man.

STUDIES IN HUMAN METABOLISM

Skin

The first studies on the possible relationship of vitamin B_6 to human metabolism were directed at its use in the treatment of skin lesions. In 1942 Jolliffe et al.[66] on their cure of adolescent acne in a group of patients. Eczema and seborrheic dermatitis were apparently controlled by vitamin B_6 in a series of patients observed by Wright et al.[67] More recently, Mueller and Vilter[68] have produced dermatitis in man using diets low in vitamin B_6 and administering deoxypyridoxine. This seborrheic dermatitis readily responds to oral or topical vitamin B_6 therapy.

Pregnancy

The relationship of vitamin B_6 to pregnancy has been under study since Willis[69] used it in the treatment of hyperemesis gravidarum in 1942. Subsequently it was demonstrated that increased fasting blood urea levels and increased plasma glutamic acid levels occur during pregnancy, particularly with the nausea and vomiting of the first trimester.[70,71] Therapy with vitamin B_6 lowered both of these to normal.

More direct evidence of the relation of vitamin B_6 to pregnancy has been brought out by Wachstein and Gudaitis[72] using the tryptophan load test to demonstrate increases in xanthurenic acid excretion. They showed an increase in xanthurenic acid excretion toward the end of the first trimester with apparent marked increases during toxemias. Pyridoxine therapy reduced the excretion of xanthurenic acid and other tryptophan excretory products.

Recently Wachstein et al.[73,74] have used the tyrosine decarboxylase technic for measuring pyridoxal phosphate content of circulating leukocytes and plasma in pregnant women at term, in umbilical cord blood, and in nonpregnant control subjects. Their data, as well as that of Chow, demonstrate high levels in the cord blood suggesting that the

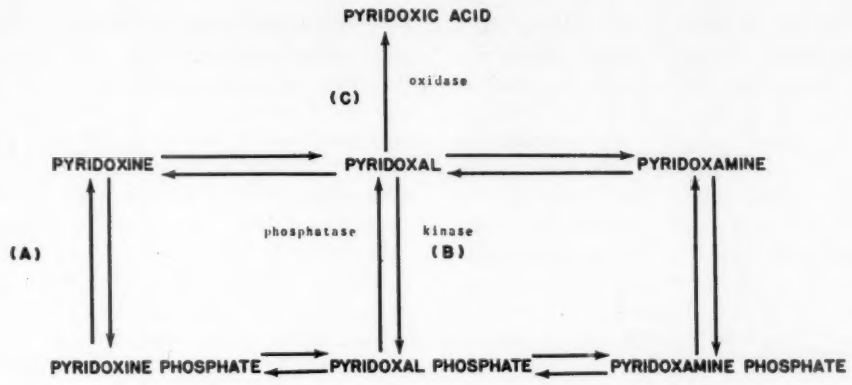


FIG. 3. The metabolism of the members of the vitamin B_6 group.

fetus is amply supplied with vitamin B_6 under normal conditions of pregnancy.[75]

Central Nervous System Metabolism

The first studies exploring the possible relationship of vitamin B_6 to human central nervous system activity were carried out empirically by Fox and Tulledge[76] with some success in the treatment of epilepsy.

The earliest demonstration of a direct relationship of vitamin B_6 deficiency to convulsive disorders in man was made by Snyderman et al.[77] Using a diet completely free of vitamin B_6 for two and a half months they were able to produce convulsive seizures in two mentally defective infants. The seizures disappeared immediately upon the administration of vitamin B_6.

In 1954, Coursin[78] and Molony et al.[79] reported convulsive seizures in infants who were receiving a particular formula preparation. Subsequent studies showed the milk to be low in vitamin B_6, containing 60 μg. per L. of pyridoxine. Clinical studies and electroencephalographic tracings clearly demonstrated the therapeutic value of pyridoxine in these cases. Follow-up studies of twenty-eight of the fifty-four infants originally described showed no recurrence of convulsive seizures or appearance of mental retardation.[80,81] Reports of similar observations have been made by Hansen,[82] May [83] and Eliot.[84]

Hunt et al.[85] have described a patient who seems to require an excessive amount of pyridoxine, necessitating a large daily intake of vitamin B_6 in order to prevent the recurrence of convulsive seizures and the development of metal retardation. Additional cases have been reported by Bessey,[86] Adam,[87] Marie,[88] Scriver,[89] and their associates. This suggests the possibility that many infants may have various forms of this syndrome as inborn errors of metabolism. In addition to these recognized problems, there is some experimental evidence suggesting a possible relationship of vitamin B_6 to mongolism,[90a] oxalosis[90b] and to phenylketonuria.[91]

Vitamin B_6 has been reported to play an important role in a number of other clinical conditions including anemia,[92] cardiac decompensation,[93] radiation effects,[94] skin grafting,[95] isoniazid therapy,[96] atherosclerosis,[97] and alcoholism.[98]

MECHANISMS PRODUCING VITAMIN B_6 DEFICIENCY

In order to provide a working hypothesis for understanding the relationships of the various members of the vitamin B_6 group, Figure 3 presents a schematic diagram that has been adapted from Snell.[99] It involves all the interrelated forms of pyridoxine, pyridoxal, pyridoxamine, and their phosphorylated derivatives. Bain[100] has shown that all these members of the vitamin B_6 group are probably rapidly converted in the intact animal into pyridoxal phosphate and pyridoxamine phosphate within two hours.

The diagram indicates that the phosphoryla-

tion of pyridoxal requires the activity of a specific enzyme, pyridoxal kinase utilizing a mole of adenosine triphosphate to produce pyridoxal-5-phosphate.[101] To reverse this reaction, a second enzyme (phosphatase) is needed to remove the phosphate, leaving free pyridoxal. Pyridoxal in turn may re-enter the same cycle or may proceed by transamination to pyridoxamine and thence through pyridoxamine phosphate by oxidation or transamination to pyridoxal phosphate. On the other hand, pyridoxal may be changed by the aldehyde oxidase of the liver to 4-pyridoxic acid.[5] This compound is an inactive degradation product and is excreted by the kidney.

There is evidence suggesting the possibility of three major areas of derangement in this over-all system. The first is concerned with the availability of vitamin B_6 to the body as indicated at A in the diagram. At this point, there may be a limitation of dietary intake of vitamin B_6 ranging from suboptimal to complete absence of the vitamin. In some experimental situations in animals, the bacterial activity of the gut may play an important role in controlling the amount of vitamin B_6 that may be forthcoming from this source.[102]

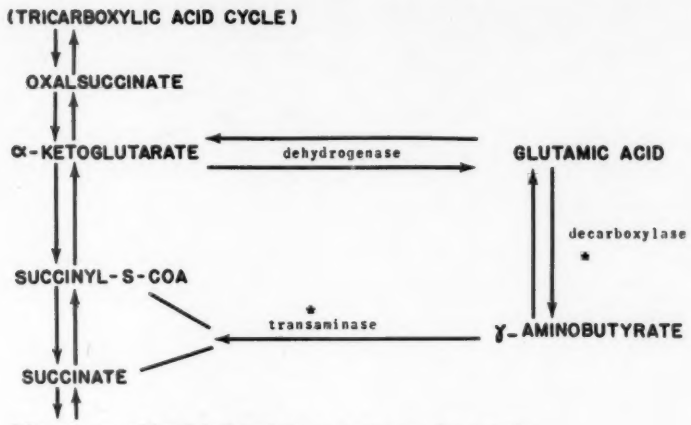
Under circumstances of low vitamin B_6 intake, the animal continues to utilize its stored supplies with gradual depletion and continued excretory losses of vitamin B_6 and pyridoxic acid. Eventually, depending upon the species, symptoms such as those previously described will become manifest.

A second group of problems centers about the possibility of the decreased availability of pyridoxal-5-phosphate for coenzyme activity as designated at B in the diagram. This may arise from a lack of adequate kinase to provide the necessary quantities of pyridoxal phosphate. Work by Mathues et al.[103] has suggested that chloroalkylphenothiazine hydrochloride may block this step, resulting in some of the effects of this drug. The administration of a hydrazide such as isoniazid has been shown to produce an inactive pyridoxal hydrazide complex which is excreted unchanged and causes the continued loss of vitamin B_6 from the body.[104] Deoxypyridoxine may also be listed in this group in that it competes with the pyridoxal phosphate that may be formed normally and successfully occupies its coenzymatic position in the enzyme reactions, thereby serving as an antivitamin and creating a deficiency effect in the presence of pyridoxal phosphate. With concurrent dietary deficiency of vitamin B_6 the action of these drugs is greatly increased.

Conceivably the pathway to pyridoxal phosphate from pyridoxamine and pyridoxamine phosphate may also be interfered with in some fashion. The capacity of this sequence of reactions to produce pyridoxal phosphate is not known, although it may supply an alternate but limited means for coenzyme formation in the presence of a block at B.

The third area for vitamin B_6 problems is at C. The abnormalities seen here are concerned with the increased formation of 4-pyridoxic acid and its excretion, or excessive losses of vitamin B_6.[105] Experimental observations in patients with evident central nervous system symptoms that are related to vitamin B_6 have revealed examples of these two mechanisms. In the first group, there is excessive conversion of vitamin B_6 to pyridoxic acid, producing a secondary vitamin B_6 deficiency resulting in recurrent convulsions. With supplementation of vitamin B_6 (100 mg.), temporary improvement of electroencephalographic and central nervous system activity extends over a period of one to one and a half hours.[106] During this time, there is rapid oxidation of administered vitamin B_6 to 4-pyridoxic acid resulting in a decrease in available pyridoxal and the urinary excretion of large amounts of pyridoxic acid.

The second mechanism is operative in those who demonstrate an inordinately high requirement for vitamin B_6. Studies in these subjects have shown excessive renal loss of pyridoxal and pyridoxamine. Despite normal blood levels of vitamin B_6, these infants exhibit rapid depletion of tissue stores leading to symptoms of central nervous system hyperirritability, abnormal electroencephalograms, convulsive seizures, coma, and possibly death. With supplementation of vitamin B_6 (75 to 100 mg.) temporary improvement of this situation occurs but the symptoms usually recur



* Requires pyridoxal-5-phosphate as coenzyme for reaction.

FIG. 4. The relationship of gamma amino butyric acid to the tricarboxylic acid cycle.

within forty-eight to seventy-two hours due to continued excessive losses by vitamin B_6.[83–87] If the patients survive, adequate pyridoxine must be administered daily to control convulsions and mental deterioration.

These patients are suffering from inborn errors of metabolism that reduce tissue supplies of pyridoxal-5-phosphate, thereby interfering with vitamin B_6-dependent enzyme systems. Furthermore, there may be an additional congenital abnormality of the particular central nervous system apoenzyme involved, resulting in its having a lower affinity for vitamin B_6. This would result in the need for a higher intake of vitamin B_6 in order to provide enough pyridoxal phosphate to force the enzyme reaction by mass action.

It is quite possible that variations of these mechanisms may occur in older children who were previously normal and then began to have convulsions and progressive mental retardation. Cochrane[107] has reported on several patients in whom pyridoxine therapy appeared specific in improving the central nervous system symptoms.

The exact mechanism through which decreased pyridoxal phosphate affects cellular metabolism is presently nuclear. A number of hypotheses have been advanced particularly with regard to the nervous system phenomena that accompany changes in vitamin B_6.

As can be seen from Figure 2, vitamin B_6 is an important coenzyme in the formation of serotonin (5-OH tryptamine) as well as norepinephrine. The recent work of Weissbach[108] has shown a relation between vitamin B_6 concentration and activity of this substance. Furthermore, Mathues' work with the effect of chloroalkylphenothiazine hydrochloride on pyridoxal kinase may lend further support to the possibility of this being an important mechanism.

The possibility that an abnormality of gamma aminobutyric acid metabolism may be the cause of the central nervous system changes during vitamin B_6 deficiency has been suggested by Killam.[109] Figure 4 shows the vitamin B_6-catalyzed metabolic pathway of glutamic acid to gamma aminobutyric acid to succinic semi-aldehyde which may provide the substrate for 20 to 40 per cent of cerebral oxidative metabolism.[110,111] Roberts et al.[112] have shown that the glutamic decarboxylase forming gamma amino butryic acid is more susceptible to pyridoxal phosphate deficiency than is the transaminase that acts with alpha ketoglutarate to dispose of it. A decrease in the coenzyme in the central nervous system might therefore block this pathway causing a decrease in cerebral oxidation resulting in convulsive seizures. In experimental studies in man, it has been reported that the ad-

ministration of gamma aminobutyric acid temporarily controlled convulsive seizures that were caused by a deficiency of vitamin B_6.[113]

The recent finding of pyridoxal phosphate in phosphorylase may also be a significant step in the over-all pattern of vitamin B_6 effect.[14] Furthermore, the relationship of vitamin B_6 to ATP formation may be a factor in energy transfer leading to clinical symptoms.[106] It must be emphasized that none of these suggestions has been demonstrated as the single mechanism of the vitamin B_6-deficiency syndrome.

Attention should also be drawn to the fact that in the intact animal there are many other enzyme systems and vitamin interrelationships that may be affected to a greater or lesser degree by the decrease in availability of pyridoxal phosphate. Keeping in mind the numerous biochemical systems in which pyridoxal phosphate serves as an important coenzyme, the scope of the problem of solving all the ramifications of this deficiency is more fully appreciated. The molecular level is quickly reached in a consideration of relative affinity of any given enzyme with regard to other enzymes requiring vitamin B_6. Furthermore, the immediate availability of pyridoxal phosphate at an interface in adequate concentration for a particular reaction would in turn limit the capacity of that reaction regardless of the particular enzyme's affinity for the pyridoxal phosphate. Since a high concentration of pyridoxal phosphate within the cell is in the mitochondria, it is quite conceivable that the intracellular distribution of the enzymes requiring it may be fortuitously placed so that they may be affected preferentially by the actual distribution of the pyridoxal phosphate within the cell.

NUTRITIONAL REQUIREMENTS

While the exact daily requirements for vitamin B_6 have not as yet been established for man, experimental data suggest that a minimum of 1.5 to 2 mg. per day may provide an adequate supply.[114,115] Patients with vitamin B_6 dependency have been shown to need at least 5 to 10 mg. or more daily.[116] With aging, there appears to be an increase in need for the vitamin, perhaps to 4 to 6 mg. per day.[117,118] Studies carried out during pregnancy have demonstrated increased needs for vitamin B_6 to 10 mg. daily. Large doses of vitamin B_6 (50 to 100 mg. per day) may be required in special situations such as radiation exposure,[94] cardiac failure,[93] and in isoniazid therapy for tuberculosis.[96] Excessive use of pyridoxine to the extent of producing toxic signs has not been described in man.

SUMMARY

The biochemical and physiologic aspects of vitamin B_6 deficiency in animals and man are briefly discussed. A schematic diagram of the possible mechanisms producing the development of vitamin B_6 deficiency in the intact animal is presented. The importance of adequate nutritional intake and vitamin B_6 therapy is stressed. Particular reference is made to the existence of convulsive disorders and mental retardation in infants as symptoms of a congenital biochemical defect that may be corrected if recognized early and adequate treatment instituted.

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Serum Magnesium and Serum Cholesterol Changes in Man

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A RELATIONSHIP between magnesium and serum cholesterol was observed in 1933 by McCollum and his associates in their classic work on magnesium deficiency in the dog.[1]

Two years ago, Bersohn and Oelofse[2] reported that South African Bantus have higher serum magnesium and lower serum cholesterol levels than Europeans. Moreover a significant negative correlation between magnesium and cholesterol levels in serum was found in a group of forty-seven Europeans. No data are given by Bersohn and Oelofse about the magnesium content of the diet eaten by their subjects.

More recent observations[3,4] failed to confirm the findings of the South African workers. Experimental work on animals indicates a possible relationship between magnesium and atherosclerosis[5–8] but no apparent effect of dietary magnesium on serum cholesterol concentration.

Controlled dietary experiments conducted in this laboratory gave an opportunity to study the changes in serum cholesterol and serum magnesium levels in different dietary situations intended to produce changes in the serum cholesterol levels with and without changes in magnesium intake.

The results of two experiments are reported.

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EXPERIMENTAL

The details of the two experiments have been reported elsewhere.[9,10] The subjects in experiment 1 were twenty-two men of forty-two to sixty-two years of age, schizophrenic residents of the Hastings State Hospital. After two weeks on a low fat (LF) diet, providing on the average 3,260 calories per day, and containing 34 gm. of fat (9.5 per cent of the total caloric intake) half of the men were fed with the experimental diet OO, and the other half were given diet SO for another two weeks. The diets were then reversed for another two-week period.

The experimental diet OO was made by substituting 100 gm. of olive oil per day for 900 cal. of simple carbohydrate in the LF diet. The mean caloric content of this diet, adjusted to maintain calorie equilibrium in these men, was 3,380 cal. per day and contained 134 gm. of fat corresponding to 36.3 per cent of the total calories.

The experimental diet SO was made by substituting 32 gm. of a mixture of 47.2 per cent safflower oil and 52.8 per cent oleostearine for 288 gm. of carbohydrate in the LF diet so as to match the OO diet in respect to saturated and polyene fats but containing 67.9 gm. less of monoene fats. The mean caloric content of this diet was 3,190 cal. per day and the total fat content 65.1 gm. corresponding to 18.3 per cent of the calories.

In experiment 2, twenty-eight subjects were used including the men in experiment 1. The duration of the experiment was sixteen weeks. In the first two weeks the men were given a controlled house diet (CHD) which corresponded to the usual American diet and had a proportion of fat calories close to 40 per cent

TABLE I
Serum Magnesium and Cholesterol Levels (mg. per 100 ml.) in Experiment 1

Men	Nov. 12–14		Nov. 26–27		Dec. 10–12	
	Magnesium	Cholesterol	Magnesium	Cholesterol	Magnesium	Cholesterol
	LF Diet		SO Diet		OO Diet	
A1	1.90	130	1.97	154	1.95	181
A2	2.22	166	2.12	170	2.24	168
A3	1.81	127	1.88	132	1.86	140
A4	2.04	182	2.12	180	2.07	190
A5	2.08	169	2.10	165	1.98	194
A6	1.84	136	1.96	142	1.90	150
A7	2.12	196	2.04	224	2.08	226
A8	2.00	187	1.97	166	2.00	199
A9	2.16	211	1.95	215	2.05	207
A10	2.26	156	2.10	140	2.17	143
A11	2.04	165	2.06	183	2.04	184
Mean	2.043	165.9	2.024	170.1	2.031	180.2
Standard error of mean	±0.044	±8.2	±0.025	±8.9	±0.034	±2.2
	LF Diet		OO Diet		SO Diet	
B1	2.18	170	1.94	186	1.98	186
B2	2.14	148	2.05	163	2.08	185
B3	1.98	139	2.05	140	1.98	131
B4	2.18	152	2.32	140	2.16	174
B5	2.24	149	2.02	148	2.09	156
B6	2.39	133	2.24	150	2.34	148
B7	2.58	156	2.28	188	2.38	208
B8	2.19	144	2.23	174	2.16	170
B9	2.00	128	2.19	142	2.01	138
B10	2.46	152	2.22	180	2.16	186
B11	2.22	190	2.04	173	2.07	212
Mean	2.233	151.0	2.144	162.2	2.128	172.2
Standard error of mean	±0.055	±5.2	±0.038	±5.7	±0.040	±8.1
Mean for 22	2.138	158.7	2.084	166.1	2.080	176.4
Standard error of mean for 22	±0.040	±4.9	±0.026	±5.2	±0.028	±4.2

NOTE: LF = low fat; SO = safflower-oleostearine; OO = olive oil.

and a mean caloric value of 3,200 cal. per day. This diet was given again for another two weeks at the end of the experiment. Two diets were used during the two experimental periods of six weeks each. The two experimental diets were equivalent to the CHD as to total caloric value, but diet I was rich in fruit, fresh vegetables, and legumes, while diet A had a higher proportion of sugar and lactose than diet I.

Both diets I and A were of lower fat content than the CHD. Each of these two diets was used at two different levels of fat content. For the object of the present paper however these fat differences will not be considered, and the results obtained with both levels of fat

TABLE II

Differences in the Magnesium and Cholesterol Levels of the Serum Corresponding to Changes in the Diet; Mean Values for Twenty-Two Men

Diet Comparison	No.	Δ Magnesium			Δ Cholesterol		
		Mean (mg. %)	Standard Error (mg. %)	P	Mean (mg. %)	Standard Error (mg. %)	P
LF *minus* OO	22	0.050	±0.028	0.09	−12.7	±3.5	0.0015
LF *minus* SO	22	0.061	±0.024	0.015	−12.7	±3.8	0.003
LF *minus* (OO + SO)	44	0.056	±0.018	0.004	−12.5	±2.9	0.001

will be considered together. The administration of the diets was made according to the usual reversal or switchback design, that is to say, half of the men received diet I and the other half diet A for six weeks, and the diets were reversed in the following period.

Blood samples for analysis of magnesium and cholesterol were taken twice at the end of three and six seeks during the experimental periods as well as at the end of the CHD periods.

METHODS

Serum cholesterol levels were measured by the Anderson and Keys modification of Abell's method.[11]

Serum magnesium levels were determined by the method of Orange and Rhein adapted to the Coleman spectrophotometer.[12]

A standard prepared by dilution of dry human plasma was used throughout. The magnesium content of this plasma was established by repeated analysis made during the duration of the experiment. The mean value of all these different analyses was taken as standard value to calculate the magnesium content of the unknown serum samples. The diets were collected and analyzed as described by Keys, Anderson and Grande.[9] The magnesium content of the diets in experiment 2 was measured by applying the method of Orange and Rhein to the ash.

RESULTS

The cholesterol and magnesium data of experiment 1 are given in Table I.

There was an increase in serum cholesterol levels when the men changed from the low fat diet to either the OO or SO diets. A small decrease in serum magnesium level was observed at the same time. The statistical analysis of the results are given in Table II. The change in the magnesium level was statistically significant when all the values for the two series of dietary comparisons (LF minus OO, and LF minus SO) were pooled (Table II).

No significant correlation was found ($r = -0.099$) between the magnesium and cholesterol values. Also no significant correlation was found between changes in cholesterol and changes in magnesium, the correlation coefficient for the forty-four pairs of changes being $r = 0.17$.

The results of experiment 2 are given in Table III. The serum cholesterol and magnesium values are the means of duplicate estimations made on four samples of serum from each man for each dietary period. The CHD values were taken at the end of the first and last CHD periods and the values for the other dietary periods correspond to samples taken after three and six weeks of subsisting on the diet (two samples on each occasion). The highest cholesterol value was observed when the men were receiving the CHD; the lowest with diet I. The mean changes in magnesium and their probability level are given in Table IV with the changes in cholesterol.

COMMENTS

The changes of magnesium content of the serum observed were small, and in most cases

TABLE III*
Serum Magnesium and Cholesterol Levels (both in mg. per 100 ml.) in Experiment 2

Subjects	Diet					
	CHD (287 mg.)		I (407 mg.)		A (360 mg.)	
	Magnesium	Cholesterol	Magnesium	Cholesterol	Magnesium	Cholesterol
W1	2.24	220	2.12	167	2.24	172
W2	2.11	200	2.02	171	2.20	181
W3	2.08	206	2.00	144	2.13	150
W4	1.98	262	1.92	200	1.97	229
W5	2.04	230	1.99	166	2.18	185
W6	2.09	178	2.04	139	2.11	160
W7	2.28	232	2.30	177	2.44	189
X1	2.07	165	2.10	120	1.96	131
X2	1.91	208	2.04	148	1.95	176
X3	2.13	197	2.18	154	2.10	184
X4	2.08	154	2.16	118	2.08	138
X5	1.88	168	2.10	126	2.03	142
X6	2.08	273	2.10	240	2.03	236
X7	2.24	174	2.28	137	2.24	156
Y1	2.20	246	2.09	185	2.24	193
Y2	1.84	205	1.91	146	1.84	159
Y3	2.32	258	2.26	206	2.46	222
Y4	2.04	181	1.98	149	2.10	166
Y5	1.95	222	1.93	183	1.93	200
Y6	2.08	234	2.04	190	2.06	234
Y7	2.04	229	2.08	198	2.02	209
Z1	2.13	200	2.12	183	2.15	209
Z2	1.82	190	1.88	139	1.87	148
Z3	2.18	234	2.34	176	2.27	204
Z4	2.01	223	2.11	174	2.02	188
Z5	2.29	195	2.29	120	2.32	158
Z6	2.21	253	2.29	194	2.27	202
Z7	2.21	231	2.26	176	2.11	190
Mean	2.090	213	2.104	165	2.118	183
Standard error of mean	±0.025	±5.87	±0.025	±5.62	±0.029	±5.54

* The magnesium content of the diet (mg. per day) is given for each diet at the top of the corresponding column CHD = controlled house diet; diet I = Italian type diet; diet A = American type diet.

TABLE IV
Changes in Serum Cholesterol and Magnesium Levels in Experiment 2; Mean Values for Twenty-Eight Men

Dietary Change	Cholesterol	Magnesium	
	Mean Change (mg. per 100 ml.)	Mean Change (mg. per 100 ml.)	P
A *minus* I	18	+0.014	0.5
CHD *minus* I	48	−0.014	0.37
CHD *minus* A	30	−0.028	0.035

not significant statistically. In the first experiment the magnesium content of the diets is considered to have been constant, since the difference in the diets consisted in the substitution of sugar for fat. In this experiment, the cholesterol changes were small, but significant and the correlation analysis showed that there was no significant association between cholesterol and magnesium changes.

In experiment 2, the changes in cholesterol were more marked (Table III) but the changes in magnesium were not significant (Table IV). The lowest level of magnesium was

observed during the CHD periods, when the cholesterol values were highest, but the highest magnesium level (diet A) did not correspond to the lowest cholesterol value.

No correlation was found between the magnesium content of the diet and the magnesium level. Diet I had the highest magnesium content (407 mg. per day), but the serum magnesium values were lower than with diet A, which had a slightly lower magnesium content (360 mg. per day).

It is known that only a small proportion of dietary magnesium is absorbed[13] and that deprivation of dietary magnesium for periods of twenty-two and twenty-seven days in man fails to affect the serum magnesium level.[14] Whether very long periods of magnesium deprivation can produce changes in serum magnesium levels is questionable.

The data of the present experiments, together with the publication of Brown et al.[3] and of Jankelson et al.[4] seem to indicate that changes in serum cholesterol levels are not associated with changes in magnesium levels.

SUMMARY

The relationship of changes in serum magnesium concentration to changes in serum cholesterol concentration was studied in controlled dietary experiments in man. Changes in serum cholesterol levels were induced by interchanging isocalorically various amounts and kinds of dietary fats and carbohydrates. In one experiment in which there was a significant increase in serum cholesterol concentration the mean serum magnesium level decreased slightly. The "within-men" correlation between magnesium and cholesterol changes was not statistically significant. In a second experiment with even greater changes in serum cholesterol levels no significant changes in serum magnesium levels were observed. It is concluded that no systematic relationship existed between changes in serum magnesium and serum cholesterol levels under the conditions of these dietary experiments.

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Biological Study of Fifty-Six–Day Protein-Free Diet in Dogs

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THIS biological study was designed to ascertain various physiologic and histopathologic changes in dogs given a basal diet of no protein but only a prepared calorie mix computed at 100 cal. per kg.[1–3]

The constituents of this calorie diet are standard in this laboratory and have been found to be palatable for dogs. In our own experience, this total number of calories per kilogram has been found to be an entirely adequate caloric intake in various investigations in which dogs have been the test animals used. The composition of the mixture is as follows:

Sucrose	2,720	gm.
Dextrin	280	gm.
Agar	40	gm.
Wesson's salt	160	gm.
Choline	12	gm.
Paba	0.6	gm.
Lard	600	gm.
Mazola	200	gm.

The final product comes to approximately 480 cal./100 gm.

DESIGN AND METHOD

Our aim was to give this preparation as the sole feeding for a period of fifty-six days, noting the gross biological and histopathologic changes during and at the end of the test period. At the termination of the fifty-six–day test period, the following surgical procedures were performed under light nembutal anesthesia: left nephrectomy and adrenalectomy, with a wedge resection of liver and partial pancreatectomy. The tissues were stained with hematoxylin and eosin and Sudan III stains. A total of twenty healthy mongrel dogs that were known to be free of parasitic gastrointestinal disease were used in the series. All these animals were housed in individual cages in an air-conditioned room for thirty days prior to the experiment.

Using seven-day intervals, various determinations were made which included: alkaline phosphatase, nonprotein nitrogen, total protein with albumin:globulin ratio, bilirubin (direct and total), prothrombin times, hemoglobin, hematocrit, clotting times (six-tube method), serum protein electrophoresis (Spinco paper electrophoretic technic), and periodic microscopic urinalysis. All urine specimens were carefully collected and saved for determining the urinary excretion of nitrogen (semi-micro Kjeldahl technic), and in conjunction with nitrogen excretion data, careful weight recordings were made daily.

As in all animal experimentation, close observation and physical inspection are extremely important and in no way secondary to the other procedures carried out. Each animal was carefully observed for gross physical changes and variations from normal activity, both in and out of their cages.

RESULTS

The observations and results are presented in the following order: gross physical changes and activity variations; histopathologic changes; blood chemistry, nitrogen balance data, weight change and electrophoretic serum protein determination. Data are presented in

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TABLE I
Results of Eight Weeks of Protein-Free Diet
(Data are Averages of Twenty Days)

Period	Bilirubin		Prothrombin Times	Alkaline Phosphatase	Non-protein Nitrogen	Proteins		Hemoglobin	Clotting Time	Weekly Urinary Excretion of Nitrogen (gm.)	Weight (kg.)
	Direct	Total				Total	Albumin				
Control	0.25	0.075	7.1	6.8	20	6.35	3.65	15.4	4′8″	14.4	16.4
1st test week	0.3	0.9	7.2	7.6	23	5.7	3.20	15.5	5′10″	12.7	16.5
2nd test week	0.5	1.1	7.6	10.0	23	5.2	2.70	14.8	6′5″	7.3	17.0
3rd test week	...	...	8.5	10.4	24	5.3	2.5	14.6	6′5″	6.0	17.0
5th test week	...	...	8.5	11.5	21	4.9	1.8	14.5	7′0″	3.8	17.5
8th test week	0.7	1.5	9.0	13.6	22	4.7	0.7	14.4	9′0″	2.9	18.0

table form as an average of the results obtained from the dogs in the series (Table I).

Gross Physical Changes and Activity Variations

In general, there was no apparent change in the physical appearance or activity in any of the animals for approximately the first four weeks, but beyond this period definite changes were observed. A progressive decrease in over-all activity was manifested in all the animals and signs of general lethargy became pronounced as the test period neared its conclusion. No attempt was made to get up or bark, which is a normal reaction at feeding time; this became a consistent observation. None of the dogs became vicious, although there was less response to petting and individual attention. The loss of the richness in the texture of the hair, with subsequent shedding, and marked erosions at the dentogingival margins were the most marked physical changes occurring in all the dogs.

Histopathologic Findings

Most distinctive were the changes in the adrenal glands (Fig. 1). The amount of capsular and subcapsular fibroplasia was surprising. At many areas along the periphery of the cortex, projections of fibrous tissue could be seen extending through the entire cortical area to the medulla along the route of small blood vessels. Lateral extensions of this fibroplasia were seen in all three cortical zones and appeared irregularly and loosely

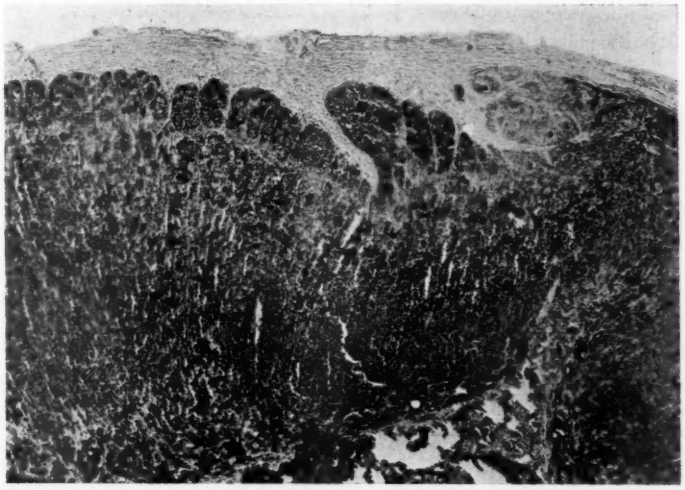

FIG. 1. Representative photomicrograph of the adrenal demonstrating excessive fibroplasia. Original magnification × 65.

arranged. The architecture of the three zones was poorly differentiated. Along the corticomedullary zone were many cells containing large clear vacuoles and scattered diffusely throughout the adrenal cortex were cells containing lipid material taking a positive stain with Sudan III and IV.

The kidney, liver and pancreas did not demonstrate architectural distortion by fibroplasia which was as pronounced in the adrenal gland. Perhaps the most singular feature in the liver was the excessive deposition of glycogen. This was more particularly seen in the cells lining the canalicula and not quite as prominent in the more central hepatic parenchyma.

The kidneys appeared to have a minimal accumulation of fat. The glomeruli within the cortex showed no apparent fat, and the proximal convoluted tubules, although dilated, also gave no evidence of fatty change. A few of the cells had a fine pink cast in the epithelium with the Sudan stain but it was not in the droplet form that is usually seen in fat deposition. In the medulla there was also considerable dilatation of the tubules but here again no apparent change took place within the epithelium itself. A few of the tubules did show occasional fine fat droplets within the lumen.

Fat deposition was also absent from the liver; however, the excessive deposition of glycogen was very notable. The hepatic cells in general showed marked vacuolization and none of these vacuoles stained positively with Sudan III or IV.

As with the liver and kidney, the pancreas had no observable fat deposition within the pancreas proper, although there were a few large fat droplets taking the Sudan stain around some of the larger vessels. The acini and islet tissue did not appear abnormal.

Hematologic Nitrogen Balance Determinations and Serum Protein Electrophoretic Results

Changes in plasma proteins became apparent by the third and fourth weeks of the experiment. There was a marked hypoproteinemia, more particularly in the albumin fraction, and a trend toward albumin:globulin ratio reversal as early as the third week.

The clotting time (six-tube technic) was progressively increased and an abnormal prothrombin time likewise became apparent. Hemoglobin concentration showed a consistent drop in all animals at the conclusion of the experiment. Red blood cell counts were also decreased by the seventh week, but peripheral smears showed no abnormal morphologic changes.

Alkaline phosphatase (expressed in Bodansky units) showed varying degrees of elevation, but its accuracy to reflect the true extent of hepatic damage or involvement has never been too readily accepted in past biological experimentation in this laboratory. Nonprotein nitrogen levels remained within normal limits and the values obtained did not reflect renal disease. There was a small increase in both the direct and total bilirubin measurements in all animals by the end of the test period.

The weights before the institution of the test diet were maintained and this can be accounted for in part by the adequate caloric intake and the progressive decrease in physical activity.

As was to be expected, urinary excretion of nitrogen became greatly reduced, and at the terminal part of the experiment, the small amount of nitrogen excreted undoubtedly represented protein breakdown from the animal's own tissues.

Electrophoretic patterns of serum proteins were determined on the tenth, twentieth, thirtieth, fortieth and fiftieth test days. There was a rapid decrease in the albumin peak. This was seen electrophoretically by the tenth test day. The *alpha*-globulins showed a decrease by the twentieth day, while the *beta*- and *gamma*-globulin fractions did show a reduction in total components by the fortieth test day.

The electrophoretic findings corresponded quite well with the blood and plasma values which were drawn at approximately corresponding times. Total proteins showed a progressive fall, initially due to a reduction in the albumins, but at the end of the fifty-six–day test period there was also a decrease in the globulin fraction.

COMMENTS

The histologic findings of minimal or no fat in the tissues and organs reviewed was a con-

sistent observation with the exception of the adrenal gland.

In contrast, an excessive glycogen deposition was seen in all of the specimens, especially in the liver. The hepatic cells which showed extreme vacuolization did not stain with Sudan III or IV.

The adrenals, histologically, gave the most marked histologic change (Fig. 1). Fibroplasia plus fat and glycogen deposition were present. Cortical cellular patterns in the three zones were so disrupted that differentiation in most instances was very difficult and hydropic degeneration with large vacuolated cells (not staining with Sudan III or IV) were present along the entire corticomedullary junctions.

CONCLUSION

The histopathologic findings would in our estimation point to a hypoadrenal state with irreversible damage to the adrenals. The animals' ability to handle general stress conditions such as disease or rapid environmental changes would seem, in view of these facts, to be greatly impaired; the diet as given causes an excessive deposition of glycogen in the tissues but little fat; the significance of the appearance of diffuse hepatocellular vacuolization aside from vacuolization caused by glycogen deposition remains to be determined; although there was not a marked increase in prothrombin times, a small but definite elevation in all determinations was observed at the conclusion of the experiment. What abnormality, if any, in liver function is reflected in this finding was unfortunately not pursued in this particular project.

ACKNOWLEDGMENT

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Human Availability Studies of Five Vitamins in Sustained Release Form

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THE most desirable method of obtaining water-soluble vitamins for human metabolic functions is by continuous intake in our foods in the manner intended by nature. In patients with known or suspected inadequate vitamin intakes or deficiencies brought about by other mechanisms,[1,2] the dietary intake of vitamins needs to be increased by supplementation. Since the metabolism and excretion of some of the water-soluble vitamins is very rapid, a number of clinicians routinely give known or suspected vitamin-deficient patients daily multiple dosages of the deficient vitamin.[3]

In an attempt to simulate multiple daily dosing of vitamins with a single dosage form of the mixture, a sustained release capsule was designed. The design of these capsules and the analytical procedure for testing them are described by Souder and Ellenbogen.[4] The *in vitro* release pattern for all the vitamins contained in the sustained release product was 10 to 35 per cent at the end of a half hour, 40 to 65 per cent at the end of two hours, 60 to 80 per cent at the end of four and a half hours, and not less than 80 per cent at the end of seven hours.

The sustained release capsules were tested physiologically against capsules containing the same amounts of vitamin in an uncoated form. The test was performed on healthy male medical students. The study was designed closely after that used originally by Melnick et al.,[5] who showed that in normal well nourished human subjects the urinary excretion of water-soluble vitamins or their derivatives is directly proportional to the quantity consumed. Melnick and his coworkers established that a linear dose-response relationship existed between urinary excretion and the dosage level of the most completely available forms of ascorbic acid, thiamine, riboflavin, and niacinamide.

The experiment was designed so that five of the ten volunteer subjects (group A) took the immediate release vitamin capsule while the other five (group B) took the sustained release capsule. Ten days after the capsules had been taken (fourteen days after the start of the experiment) patients in the two groups were crossed over so that those in group A took the sustained release capsule while those in group B took the immediate release capsule.

All volunteer subjects were maintained on a basal diet similar to that used by Melnick et al.[5] for three days prior to the administration of the test capsules and for the thirty-six hours of urine collection following dosage with the vitamin capsules. Meals were prepared at the hospital and the test subjects ate their meals in a group. No snacks or other vitamin preparations were allowed during the experimental periods nor were the subjects permitted to smoke.

The following schedule was followed in running the test: All ten volunteer subjects were placed on the basal diet for three days. At noon on the fourth day, five men received a single capsule of sustained release vitamins (group B), and the remaining five received a

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single capsule of the immediate release vitamins (group A).

From noon of the third day, after urine was voided and discarded, to noon of the fourth day a twenty-four hour basal urine sample was collected. From noon of the fourth day, when the vitamin capsules were taken immediately before the noon meal, urine samples were collected every three hours for twelve hours and then every twelve hours for thirty-six hours. All urine samples were collected over enough hydrochloric acid to give a final concentration of approximately 0.1 normal; 100 ml. aliquots were taken from each sample period and immediately frozen.

During the interval between the end of the urine collections (thirty-six hours after the administration of the vitamins) and the start of the second basal diet period (four days before the second administration of vitamins), the volunteer subjects were allowed to return to their usual diets.

Two weeks after the start of the first experiment the "crossover" experiment was conducted in exactly the same manner as the first experiment, except that the patients in group A, who had been given capsules of immediate release vitamins, were now given the sustained release vitamins, and those in group B, who had been given capsules of sustained release vitamins, were now given the immediate release vitamins. This procedure permitted each subject to serve as his own control, while the crossover procedure helped to balance the effects of dietary or laboratory procedures for each of the two experimental periods.

ANALYTICAL PROCEDURES

The urine samples were analyzed for thiamine, riboflavin, ascorbic acid, niacin, and pyridoxine.

Thiamine was determined by oxidation to thiochrome which was measured fluorometrically.[6] Riboflavin was also determined fluorometrically.[6]

Ascorbic acid was determined colorimetrically by the method of Roe and Kuether.[7]

Niacin was ascertained by determining the concentration of the two major metabolites of this vitamin, N′-methyl-3-carboxylamide-6-pyridone and N′-methyl nicotinamide. The pyridone was determined by an ultraviolet procedure utilizing a combination of the methods of Rosen, Perlzweig and Leder[8] and Price.[9] The N′-methyl nicotinamide was determined fluorometrically utilizing the condensation reaction of Huff and Perlzweig.[10]

Pyridoxine was determined by analyzing the urine for 4-pyridoxic acid, which is reported to represent approximately 96 per cent of the urinary excretion product from this vitamin.[11] This metabolite was determined by the fluorometric procedure of Huff and Perlzweig as modified by Sarett.[12]

VITAMIN CAPSULES

The two vitamin formulations, the sustained release capsule (multivitamin "Spansule" capsule*) and the immediate release vitamin capsule (powdered or crystalline vitamins) contained the following vitamins in each capsule: ascorbic acid, 167 mg.; thiamine (as mononitrate salt, U.S.P.), 6.9 mg.; riboflavin (as 5′-phosphate sodium salt), 6.8 mg.; niacinamide, U.S.P., 68.8 mg.; pyridoxine (as hydrochloride salt, U.S.P.), 6.6 mg.; pantothenic acid (as dl-panthenol), 3.38 mg.; cyanocobalamin (crystalline, U.S.P.), 13.9 μg.; vitamin A (as palmitate ester), 16,500 U.S.P. units; and vitamin D, 1,500 U.S.P. units.

RESULTS

The average urinary excretion of each vitamin for the whole group of ten subjects on the immediate release preparation and on the sustained release preparation, and evaluations of the significance of the differences in urinary excretion of vitamins from the two formulations studied, are presented in Table I.

From these values the availability of the sustained release preparation can be calculated on the basis of 100 per cent availability for the immediate release vitamin sample. The for-

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* Fortespan,® Smith Kline & French Laboratories.

TABLE

Average Urinary Vitamin Excretion Values for Ten
(mg. of vitamin

Vitamin	Preparation (release)	Pre-Vitamin (control 24 hours)	Post-Vitamin (hours after	
			0–3	3–6
Riboflavin	Immediate	1.39	1.70	1.27
	Sustained	1.22	0.57	1.41
		Difference*	−1.13±0.23	0.14±0.15
		P†	‡	N.S.
Pyridoxine	Immediate	2.07	0.52	0.65
	Sustained	1.79	0.29	0.71
		Difference*	−0.23±0.07	0.06±0.11
		P†	‡	N.S.
Thiamine	Immediate	0.38	0.26	0.35
	Sustained	0.31	0.14	0.55
		Difference*	−0.12±0.03	0.20±0.08
		P†	‡	N.S.
Niacin	Immediate	21.7	12.2	13.7
	Sustained	20.1	5.5	12.8
		Difference*	−6.7±1.56	−0.9±0.84
		P†	‡	N.S.
Ascorbic acid	Immediate	19.1	12.5	17.1
	Sustained	17.8	6.9	17.3
		Difference*	−5.6±3.3	0.2 ±4.5
		P†	N.S.	N.S.

NOTE: N.S. $P > 0.05$ nonsignificant.

* Average quantitative difference in vitamin excreted during period indicated and standard error of the mean difference; negative signs used when vitamin excretion from immediate release form is greater than from sustained release form of vitamins administered.

mula used for these calculations over a specified time period is as follows:

$$\frac{\text{mg. excreted from sustained release} - \text{mg. excreted from control}}{\text{mg. excreted from immediate release} - \text{mg. excreted from control}} \times 100 = \text{per cent availability.}$$

The cumulative results of these calculations for each vitamin at the end of twelve, twenty-four- and thirty-six-hour periods are presented in Table II.

The logarithms of the urinary excretions (minus the calculated control value for each period) are presented as curves for the first twelve hours (Figs. 1 through 5). Control values for each three-hour period were calculated to be one-eighth the amount of vitamins excreted in the twenty-four-hour urine specimen collected before the test.

COMMENTS

Urinary excretion results are interpreted to be a reflection of the level of absorption of the vitamins. The results of Melnick et al.,[5] in which urinary vitamin levels increased linearly in the well fed healthy person as the intake of the vitamins increased, helps to substantiate this conclusion.

Urinary excretion studies are usually presented as "percentage availability" according to the method of calculation shown in Table II. Our excretion data are also presented as differences in vitamins excreted after ingestion of the two test forms (Table I) which are compared by an analysis appropriate to our cross-over design. The differences in vitamin excretions in Table I are based on the assumption that baseline (control) excretion values are equal for the two groups during any time period. The twenty-four-hour control periods before the administration of vitamins revealed no significant differences between the two test groups for any of the five vitamins tested.

I
Human Subjects Obtained in Crossover Study
per collection period)

Levels (mg.) administration)		Total Urinary Excretion (mg.) (hours after administration)		
6–9	9–12	0–12	0–24	0–36
0.61	0.29	3.87	5.31	6.36
0.84	0.41	3.23	4.56	5.68
0.23±0.09	0.12±0.04	−0.64±0.27	−0.75±0.36	−0.68±0.49
§	§	§	N.S.	N.S.
0.56	0.27	2.00	3.09	4.24
0.62	0.55	2.17	3.51	4.54
0.06±0.09	0.28±0.04	0.16±0.19	0.41±0.25	0.30±0.28
N.S.	‡	N.S.	N.S.	N.S.
0.17	0.08	0.86	1.15	1.41
0.34	0.16	1.19	1.51	1.80
0.17±0.05	0.08±0.02	0.33±0.10	0.36±0.13	0.39±0.43
‡	‡	§	§	N.S.
10.6	5.7	42.2	64.3	79.7
9.5	7.3	35.1	58.6	74.0
−1.1±0.99	1.6±0.57	−7.1±1.5	−5.8±1.4	−5.7±2.2
N.S.	§	‡	‡	§
11.9	4.8	46.3	72.4	95.8
16.7	9.0	49.9	67.2	91.2
4.8±3.8	4.2±3.1	3.6±14.2	−5.2±14.2	−4.6±18.0
N.S.	N.S.	N.S.	N.S.	N.S.

† "P" calculated as two-sided test of significance.
‡ $P \leq 0.01$ highly significant.
§ $P \leq 0.05$ significant.

In Table II are presented the twenty-four-hour vitamin availabilities which have been calculated directly from excretion data. Availability calculations for twelve- and thirty-six-hour collections are included for interest only and are based respectively on estimated control values calculated as a half and one and a half times the twenty-four-hour control urinary excretions. Statistical studies of the twenty-four-hour availabilities reveal that of the five vitamins studied only thiamine availability from the sustained release capsules differed from the immediate release form ($P < 0.01$).

The availability data (Table II) reveal that all the vitamins studied are equally or more completely available from the sustained release capsule than from the immediate release. Riboflavin, niacin, and pyridoxine availabilities show some increase between twelve and twenty-four hours which would seem to be a reflection of further sustained release

TABLE II

Percentage Availability* (Comparison of Sustained Release to Control) of Vitamins in Sustained Release Capsule as Measured by Urinary Excretion Studies

Vitamin	Availability (%) for Time Urine is Collected		
	12 hr.†	24 hr.	36 hr.‡
Riboflavin	82.6	85.2	90.0
Pyridoxine	132.3	168.6	163.4
Thiamine	153.7	155.8	158.9
Niacin	80.2	90.4	93.0
Ascorbic acid	111.7	92.7	96.0

* $\dfrac{\text{mg. excreted from sustained release} - \text{mg. excreted from control}}{\text{mg. excreted from immediate release} - \text{mg. excreted from control}} \times 100.$

† Excretion for twelve-hour control period calculated as one-half previtamin control twenty-four-hour excretion level.

‡ Excretion for thirty-six-hour control period calculated as 1.5 × previtamin control twenty-four-hour excretion level.

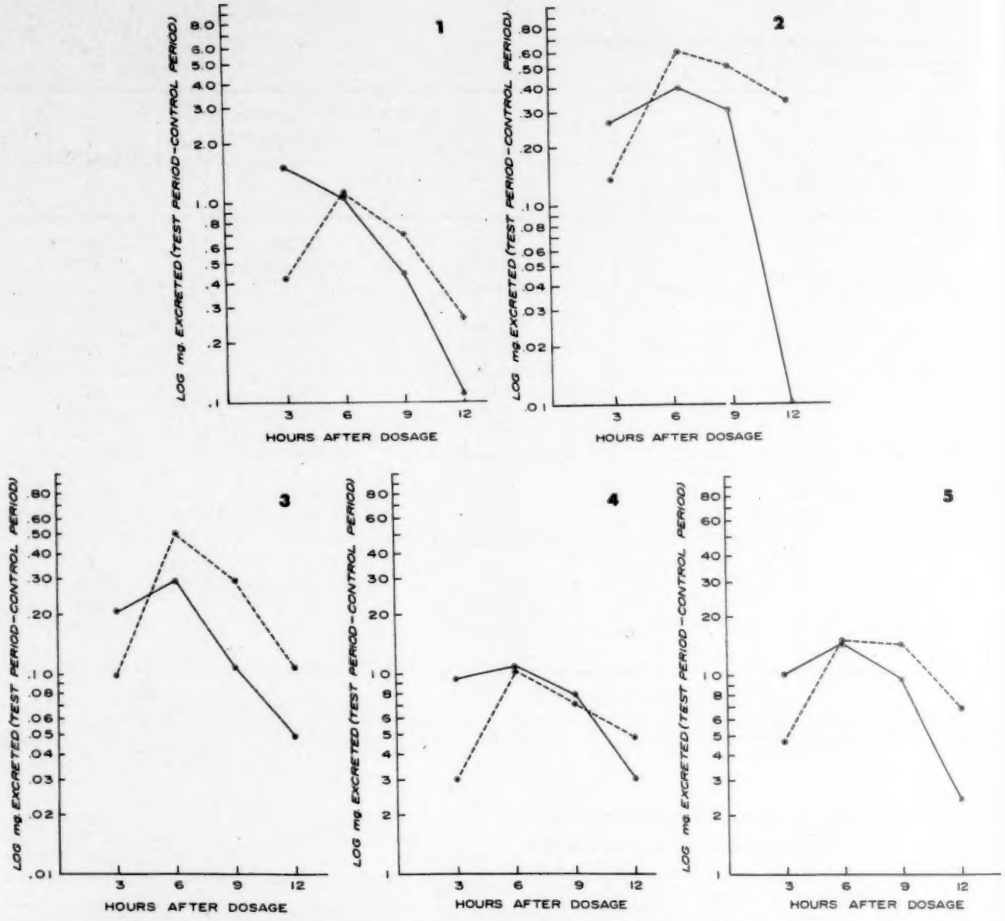

FIG. 1. Riboflavin. Twelve-hour urinary excretion patterns after immediate release ——— or sustained release - - - - vitamin dosages.

FIG. 2. Pyridoxine. Twelve-hour urinary excretion patterns after immediate release ——— or sustained release - - - - vitamin dosages.

FIG. 3. Thiamine. Twelve-hour urinary excretion patterns after immediate release ——— or sustained release - - - - vitamin dosages.

FIG. 4. Niacin. Twelve-hour urinary excretion patterns after immediate release ——— or sustained release - - - - vitamin dosages.

FIG. 5. Ascorbic acid. Twelve-hour urinary excretion patterns after immediate release ——— or sustained release - - - - vitamin dosages.

effect. The stabilization of values, except for riboflavin which continued to increase after twenty-four hours, indicates that this action is no longer operational after this time.

Data published by Friedemann et al.[13] concerning the absorption of orally administered thiamine to man offers a logical explanation for our finding that the absorption of thiamine in the sustained release form is significantly increased ($P<0.05$ as calculated in Table I and <0.01 as calculated in Table II)

over that obtained from a single dose of immediate release thiamine. These authors found that thiamine is poorly absorbed in man and that the maximum which could be taken orally without an increase in the fecal excretion of thiamine was slightly less than 2 mg. at each meal or single ingestion. Since, as these authors indicate, there seems to be a very efficient barrier mechanism for thiamine absorption in the intestinal tract, the administration of thiamine in the sustained release form may help overcome this barrier by presenting the vitamin for absorption more slowly and thereby permitting greater absorption than from a single dose of immediate release vitamins.

The explanation for the increased availability of pyridoxine from the sustained release form of vitamins is not readily apparent since pyridoxine is reported to be easily absorbable.[14] Whether the increased availability of pyridoxine (only approaching significance in both tables) is as real as that of thiamine seems to be, or is a reflection of alteration of the percentage of 4-pyridoxic acid formed and excreted in the urine, cannot be judged from these data.

The results of average urinary excretion levels of the vitamins reported in Table I clearly show that larger amounts of the vitamins were excreted into the urine by the subjects on the immediate release vitamin capsule than by those on the sustained release vitamin capsule during the first three hours of urine collection. Conversely, during the nine- to twelve-hour collection period larger amounts of vitamins were excreted by those receiving the sustained release capsules than by those receiving the immediate release vitamin capsules.

The urinary excretions of the five vitamins tested are plotted as semilogarithmic curves, as suggested by Swintosky et al.[15] (Figs. 1 through 5) and are based on the three-hour collection periods from the time the capsules were taken to the end of the first twelve hours thereafter. Similar patterns of urinary excretion were exhibited by all five of the vitamins studied (riboflavin, pyridoxine, thiamine, niacin, and ascorbic acid).

The urinary excretion data are presented with a statistical analysis for differences in excretions of vitamins for each three-hour period from zero to twelve hours and for total excretion of zero to twelve, zero to twenty-four and zero to thirty-six hours (Table I). These data show clearly that significantly more riboflavin, pyridoxine, thiamine, and niacin, but not ascorbic acid, were excreted during the first three hours by those who took the immediate release capsules than by those who took the sustained release capsules. Conversely, during the last three-hour period (nine to twelve hours) significantly more riboflavin, pyridoxine, thiamine, and niacin, but not ascorbic acid, were excreted by those receiving the sustained release capsules than by those receiving the immediate release vitamin capsules. These results indicate a significant sustained release effect for four of the five vitamins measured and at least equal availability of ascorbic acid from the two vitamin forms (immediate release and sustained release). Although niacin was calculated to be "statistically" more available from the immediate release form of the vitamin than from the sustained release form (as calculated from Table I but not from Table II), this effect was due to the low degree of variability of patient to patient results and is of little practical importance since at the end of twenty-four hours the average urinary excretion levels of niacin from the immediate and sustained release vitamin capsules were 64.3 and 58.6 mg., respectively.

Extremely high standard deviations of urinary levels of ascorbic acid made evaluation of any sustained release effects of this vitamin impossible. Four of the ten subjects failed to show any appreciable increase in urinary excretion levels of ascorbic acid on either the immediate or sustained release forms of the vitamin. Subsequent studies in this laboratory have shown that saturation of the test subjects with ascorbic acid was probably not attained on the diet used. In order to use urinary excretion as an adequate index of ascorbic acid absorption, preliminary tests should be used to show that experimental subjects are saturated with the vitamin. In view of our re-

sults with ascorbic acid, special attention should be paid to the degree of saturation of test subjects on any vitamin assayed for availability by the method used here.

Careful control of experimental variations have made statistical analyses of these data possible; however, in a relatively small population study, such as this, data should be judged more by their practical or medical significance than by the results of statistical tests. The over-all results of these studies reveal that sustained release of vitamins is possible and that the availability of vitamins from the sustained release form are comparable, and in the case of thiamine, superior to that of the immediate release form.

SUMMARY

The availability and pattern of absorption of five vitamins, as measured by urinary excretion (thiamine, riboflavin, ascorbic acid, niacin, and pyridoxine) from immediate and sustained release forms, were studied in a crossover experiment with ten healthy human subjects.

Based on a twenty-four-hour urine collection the availabilities of sustained release vitamins were calculated as the following percentages of those from the immediate release formulation: riboflavin, 85.2; pyridoxine, 168.6; thiamine, 155.8; niacin, 90.4; and ascorbic acid, 92.7.

All vitamins except ascorbic acid showed significant sustained release effects when administered in a sustained release formulation and compared with equal amounts of vitamins administered in an immediate release form. Ascorbic acid studies suffered from a lack of adequate saturation of the test subjects with the basal diet used.

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Lysine and Potassium Supplementation of Wheat Protein

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IN MANY areas of the world, protein of good nutritional quality is available in only limited quantities. If the proteins available, especially those of vegetable origin, could be made more adequate for human use, some improvement in world protein nutrition would result.

Studies of population groups such as those of Orr and Gilks[1] and of McCarrison,[2] comparing "meat eaters" to "cereal eaters," have shown an overall superiority in health and development of those consuming diets high in animal protein. These differences appeared most obvious in the growing child. Retardation of growth after the first half year of life is observed among the native infants and children of Africa, where the deficiency disease known as "kwashiorkor" is widespread and low-quality protein sources are dietary staples.[3]

The studies of Block and Mitchell[4] demonstrated that with few exceptions the biologic value of a protein is determined by the amount and relative proportions of the essential amino acids which it supplies. Although wheat contains sufficient total protein (12 per cent of the calories), less than a half is available to the animal for the formation of new tissue.[4] The recognized protein inadequacy of wheat flour is, therefore, not the result of a deficiency of protein per calorie, but of its low biologic value.

As early as 1914, Osborne and Mendel[5] found that the protein quality of wheat could be improved by the addition of lysine. This work has been subsequently confirmed by Mitchell and Smuts,[6] and Rosenberg and Rohdenburg.[7] However, Sure[8–10] reported that the addition of threonine along with lysine to whole or milled wheat flour had an even more beneficial effect on growth and protein efficiency in rats.

Differences in results of studies of lysine supplementation may be the result of biologic unavailability of lysine in some of the diets used. It is well known that prolonged heating of lysine makes this amino acid unavailable[11] for utilization—the "browning reaction." Bone growth may be stopped[12] when heated casein is the only source of protein. This is apparently self-limiting and is corrected after prolonged feeding of casein, possibly by the changing protein requirements with advancing age. Furthermore, lysine is most effective as a supplement when given mixed in the diet, and less effective when given daily, as with some vitamin preparations. For example, Vohra and Kratzer[13] found that lysine given as a single dose was not as effective as lysine given mixed in the diet as measured by growth and feather pigmentation in young turkeys.

Although many studies indicate that benefit may be obtained by lysine supplementation as far as rate of growth of young rats is concerned, the value of lysine supplementation of wheat protein has not yet been clearly demonstrated

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TABLE I
Daily Dietary Details

Baby No.	Age (mo.)	Weight (kg.)	Wheat (gm.)	Milk* (ml.)	Sugar (gm.)	Starch (gm.)	Calories/ kg.	Approximate Protein/ kg. (gm.)	Calculated† Amounts of Lysine		
									Gram Added‡	Unsupplemented (mg./ kg./day)	Supplemented (mg./ kg./day)
1	12	6.0	42	28	20	50	75	1.2	0.15	44	63
2	10	7.0	50	32	23	58	75	1.2	0.18	44	64
3	11	6.8	70	45	8	47	75	1.7	0.25	61	91
4	10	6.3	62	40	8	40	75	1.7	0.22	64	87
5	12	5.1	75	50	10	..	75	2.6	0.26	94	134
6	10	6.0	90	60	12	..	75	2.6	0.32	94	133
				200	40	..	75	2.6	....	180	...
7	14	7.0	100	75	16	..	75	2.5	0.35	95	135
			150	75	16	..	100	3.4	0.53	110	171
8	8	5.1	108	55	14	..	100	3.4	0.38	110	170
9	12	6.7	115	120	23	..	100	3.5	0.40	139	187
10	11	7.2	115	120	25	..	100	3.5	0.40	134	179
			...	315	60	..	100	3.5	....	236	...
11	10	5.9	100	110	23	..	100	3.5	0.35	144	182
12	12	6.5	108	..	25	..	75	2.0	0.38	47	99
13	9	5.8	170	..	..	..	100	3.5	0.60	69	152
14	4	4.9	167	..	..	..	120	4.0	0.60	80	178
15	4	4.2	129	..	..	..	120	4.0	0.45	85	171
16	17	7.7	130	..	33	..	75	2.0	0.45	45	92
17	15	7.3	120	..	78	..	100	2.0	0.42	39	85
18	10	7.0	120	..	70	..	100	2.0	0.42	41	89
19	9	7.1	120	..	75	..	100	2.0	0.42	39	86
20	8	5.5	92	..	55	..	100	2.0	0.32	40	87
				270	55	65	100	2.0	....	145	..
21	8	6.1	100	..	63	..	100	2.0	0.35	41	87
				350	63	64	100	2.0	....	160	..
22	10	5.5	105	..	20	..	100	2.5	0.47	54	122

* Evaporated milk was used in babies No. 1 to 11 and fresh skim milk was used with babies No. 20 and 21.
† Calculated on basis of 134 mg./gm. nitrogen in Cream of Wheat as determined by Lysine Div., E. I. du Pont Co.
‡ As lysine monohydrochloride.

in man. Since most of the information relating to amino acid supplementation is derived from animal experiments, the possibility of creating an amino acid imbalance must be seriously considered.

The present investigation was undertaken to study the value of wheat, wheat supplemented with milk and wheat supplemented with lysine in comparison with milk. The studies were carried out by the nitrogen balance technic in twenty-two infants.

While evaluating the effects of lysine supplementation of wheat on nitrogen retention it was noted that signs and symptoms of hypokalemia developed in one of the babies. Known effects of lysine-potassium interchangeability[14] were not the sole source of this potassium deficiency, as minimal signs of potassium deficiency were noted when the wheat diet was not supplemented with lysine. Potassium balances, therefore, were studied in fourteen of the twenty-two babies.

METHODS AND MATERIALS

Subjects and Procedures for Metabolic Balance Studies

The subjects were twenty-two male, underweight, Latin-American infants, age three to seventeen months, who were admitted to the

Driscoll Foundation Children's Hospital because of severe diarrhea. The diarrhea had been brought under control by the usual methods including fluid and electrolyte therapy, the administration of antibiotics when indicated, and gradual oral alimentation. All infants had been well and taking milk and other good quality proteins for not less than ten days preceding the beginning of the experimental diet. The infants were then transferred to an air-conditioned metabolic unit with almost constant temperature (76°c.) under special nursing care. Five to seven days after starting the experimental diet, and also two days after the experience of being in restraint, the balance study was begun.

Balances for each experimental diet were carried out for nine days. The duration of study for each subject ranged from three periods (twenty-seven days) to six periods (fifty-four days). Urine and feces specimens were collected separately with the aid of a modification of apparatus previously described.[15] Toluene was used as a preservative for each twenty-four-hour urine specimen which was then refrigerated. Stools were refrigerated immediately after collection. One milliliter of 10 per cent carmine was given with the first morning feeding as a stool marker every seventy-two hours. Chemical determinations were made in pooled seventy-two-hour specimens of both urine and feces.

Body weights were recorded daily. The blood measurements outlined subsequently were determined at the beginning of the study and the end of each period.

Diets

At the start of the study, no sharply defined parameters of calories, protein, carbohydrate, or fat were available for the evaluation of proteins and supplements for human infants by balance technics. Various levels of calories and protein as well as additions of milk were, therefore, fed to the infants until consistent effects were noted in several babies fed one type of diet.

The wheat used for the basic diet was commercial Cream of Wheat. The calorie and protein (calculated as nitrogen × 6.25) intake as well as the composition of the diet used for each subject is summarized in Table 1. Daily caloric intake varied from 75 to 125 calories/kg., and protein from 1.2 to 4 gm./kg. Carbohydrate calories were added in the form of sucrose or corn starch.

Wheat, sugar, and starch were weighed with an accuracy of ±0.1 gm. Water and milk were measured in a graduated cylinder of a size suitable for the amount measured. Wheat was cooked for eight minutes after boiling had started in a quantity of water sufficient to supply the infant with the calculated intake of 125 to 150 ml./kg./day. Lysine was added as l-lysine monohydrochloride, in an amount calculated to yield a total content of 0.55 per cent of the wheat, giving a lysine:tryptophane ratio of 4:1 in the wheat; 0.35 gm. lysine monohydrochloride (0.28 gm. lysine base) was added to each 100 gm. of wheat in the formula. The control for added lysine was glycine in the same nitrogen equivalent. Both lysine and glycine were weighed on an analytical balance accurate to ±0.1 mg. Lysine and glycine were added to the wheat before boiling. Potassium and sodium chloride were supplemented as 5 per cent solutions.

The wheat mixture was fed to the infants by spoon. The number of feedings varied from four to five daily, and every subject took and retained all the feedings offered. A multiple vitamin mixture (Poly-vi-sol® 0.6 ml.) was given to each infant daily.

Methods of Analysis

All determinations in this study were made in duplicate and the results accepted only when determinations agree within 3 per cent. The calorie contents of the food were calculated from standard food composition tables.[16] Nitrogen levels in the diet, urine, and feces were determined by the micro-Kjeldahl method.[17] Potassium and sodium levels were determined using a Coleman model 21 Flame Photometer.

The raw Cream of Wheat required for each subject for the total period of the study was mixed well and the nitrogen content determined. Nitrogen content in milk was determined for each three-day period after pooling.

TABLE II

Daily Balance Data: Wheat Supplemented with Milk and Lysine

Baby No.	Period	Diet*	Nitrogen				Nitrogen Balance† / Nitrogen Absorbed	Per Cent of Nitrogen Absorbed	Average Weight Change (gm./day)
			Intake (mg./kg.)	Urine (mg./kg.)	Stool (mg./kg.)	Average Balance (mg./kg.)			
1	I	WL	188.5	173.0	27.4	−11.9	−0.07	85	+11.2
	II	W	187.5	153.5	29.0	+5.0	+0.03	84	−3.2
	III	WL	188.0	155.5	25.6	+6.9	+0.04	86	−8.0
	IV	WK‡	187.0	146.0	27.2	+13.8	+0.09	85	+8.0
	V	WLK‡	192.5	144.0	28.5	+20.0	+0.12	85	0
	VI	WLK‡	195.5	146.0	25.5	+24.0	+0.14	87	+2.4
2	I	W	187.0	165.0	37.4	−15.4	−0.11	80	−4.8
	II	WL	190.5	161.0	39.6	−10.3	−0.07	79	−12.8
	III	W	192.5	151.0	34.0	+7.5	+0.05	82	−3.2
	IV	WLK‡	200.0	146.5	31.1	+22.4	+0.13	84	0
	V	WK‡	201.0	157.0	29.6	+14.4	+0.09	85	+3.2
3	I	WL	270.0	264.0	32.4	−26.4	−0.12	88	+22.4
	II	W	269.0	212.0	31.6	+25.4	+0.10	88	+1.6
	III	WL	269.0	206.0	30.2	+32.8	+0.14	89	+3.2
	IV	W	270.0	227.0	37.6	+5.4	+0.03	86	−6.4
4	I	W	262.0	216.0	51.8	−5.8	−0.02	80	+14.4
	II	WL	260.0	217.0	46.4	−3.4	−0.02	82	+4.8
	III	W	263.0	210.0	40.2	+12.8	+0.06	85	0
5	I	WL	427.0	374.5	46.3	+6.2	+0.02	89	+16.0
	II	W	430.0	390.0	40.0	0	0	91	−4.8
	III	WL	430.0	349.0	38.0	+43.0	+0.11	91	+4.8
6	I	W	376.0	308.0	53.3	+14.7	+0.05	86	+4.8
	II	WL	372.0	302.0	41.3	+28.7	+0.09	89	+8.0
	III	W	368.0	301.0	32.6	+34.4	+0.10	91	+19.2
	IV	M	421.0	325.0	28.6	+67.4	+0.17	93	−33.5
	V	W	402.0	306.0	27.7	+68.3	+0.18	93	+30.3
7	I	WL	404.0	370.0	32.7	−1.3	−0.004	92	+6.4
	II	W	399.0	370.0	32.8	+3.8	+0.008	92	−9.6
	III	W	514.0	388.0	43.5	+82.5	+0.18	92	+55.8
	IV	WL	512.0	434.0	43.8	+34.2	+0.07	92	+4.8
8	I	W	517.0	400.0	60.6	+56.4	+0.12	88	+16.0
	II	WL	523.0	407.0	67.7	+48.3	+0.11	87	+8.0
	III	W	505.0	389.0	77.7	+38.3	+0.09	85	+14.3
9	I	W	516.0	447.0	37.9	+31.1	+0.06	93	+23.9
	II	WL	516.0	384.0	38.8	+93.2	+0.20	93	+14.4
	III	W	491.0	435.0	34.7	+21.3	+0.05	93	+27.1
10	I	W	538.0	406.0	54.2	+77.8	+0.16	90	+22.3
	II	WL	528.0	415.0	48.6	+64.4	+0.13	91	+16.7
	III	W	507.0	400.0	42.1	+64.9	+0.14	92	+7.9
	IV	M	506.0	423.0	41.1	+41.9	+0.09	92	−3.2
	V	ML	503.0	394.0	43.5	+65.5	+0.14	91	−1.6
11	I	W	543.0	473.0	51.8	+10.2	+0.04	90	+9.6
	II	W	528.0	414.0	42.2	+71.8	+0.15	92	+24.0
	III	W	509.0	410.0	36.6	+62.4	+0.13	93	0
	IV	WL	540.0	402.0	41.0	+97.0	+0.19	92	+20.7
	V	W	523.0	393.0	33.6	+96.4	+0.20	94	+19.1

* See footnotes on opposite page.

TABLE II *Continued*

Baby No.	Period	Diet*	Nitrogen				Nitrogen Balance† / Nitrogen Absorbed	Per Cent of Nitrogen Absorbed	Average Weight Change (gm./day)
			Intake (mg./kg.)	Urine (mg./kg.)	Stool (mg./kg.)	Average Balance (mg./kg.)			
12	I	WL	342.0	280.0	34.5	+27.5	+0.09	90	+6.4
	II	W	342.0	273.0	34.5	+34.5	+0.11	90	−3.2
	III	WLK‡	340.0	238.0	25.0	+77.0	+0.25	97	−3.2
	IV	WK‡	342.0	252.0	25.1	+64.9	+0.20	97	−3.2
	V	WLK‡	343.0	280.0	23.6	+39.4	+0.12	98	+6.4
13	I	WLK‡	513.0	299.0	95.2	+118.8	+0.28	82	+49.5
	II	WK‡	490.0	339.0	69.3	+81.7	+0.20	86	−8.0
	III	WLK‡	500.0	372.0	54.3	+73.7	+0.17	89	−1.6
14	I	W	595.0	388.0	155.0	+52.0	+0.12	74	+9.5
	II	WL	620.0	363.0	204.0	+53.0	+0.13	67	−40.7
	III	WK‡	595.5	371.0	153.0	+71.0	+0.16	74	−14.3
15	I	WK‡	637.0	402.0	124.0	+111.0	+0.21	80	0
	II	WLK‡	657.0	406.0	152.0	+99.0	+0.20	77	−12.8
	III	WK‡	647.0	443.0	160.0	+44.0	+0.09	75	+8.0
16	I	WK	313.5	159.5	82.3	+71.7	+0.31	74	−19.2
	II	WLK	315.5	123.3	61.7	+130.5	+0.51	81	−6.4
	III	WK	317.0	140.2	72.3	+104.5	+0.42	77	0
	IV	WL	311.0	160.5	110.0	+40.5	+0.20	65	+3.2
	V	W	316.0	181.0	145.0	−10.0	−0.06	54	−30.3
17	I	WLK	293.0	128.0	22.8	+142.2	+0.53	92	+30.3
	II	WK	287.0	148.0	24.6	+114.4	+0.45	92	+3.2
	III	WLK	282.0	158.0	29.0	+95.0	+0.38	90	+19.2
18	I	WK	309.0	206.0	35.7	+67.3	+0.25	88	+23.9
	II	WLK	302.0	173.5	45.2	+83.3	+0.32	85	+22.4
	III	WK	293.0	214.0	35.6	+43.4	+0.17	88	−22.4
	IV	WLK‡	286.0	203.0	33.9	+49.1	+0.19	88	+19.2
	V	WK‡	280.0	199.0	34.0	+47.0	+0.19	88	+16.0
19	I	WLK§	294.0	186.0	67.0	+41.0	+0.18	77	+8.0
	II	WK§	292.0	195.0	73.0	+24.0	+0.11	75	+11.2
	III	WLK	285.0	170.0	56.0	+59.0	+0.26	80	+24.0
	IV	WK	281.0	195.0	44.0	+42.0	+0.18	84	−1.6
	V	WLK	278.0	148.0	40.0	+90.0	+0.38	86	+25.6
20	I	WLK	302.0	192.5	48.5	+61.0	+0.24	84	+8.0
	II	M	278.0	172.0	32.6	+73.4	+0.30	88	+11.2
	III	WLK	290.0	178.0	41.2	+70.8	+0.28	86	+14.4
	IV	M	268.0	156.0	33.8	+78.2	+0.33	87	+11.2
	V	WK	281.0	205.0	35.1	+40.9	+0.17	88	+12.8
21	I	M	306.0	203.5	25.3	+77.2	+0.28	92	+6.4
	II	WLK	253.0	166.2	30.3	+56.5	+0.25	88	+17.5
	III	M	293.5	182.0	25.0	+86.5	+0.32	92	+17.5
	IV	WK	242.5	190.5	31.5	+20.5	+0.10	87	+12.8
22	I	WK	404.0	279.0	57.5	+67.5	+0.20	86	+20.7
	II	WLK	397.0	234.0	71.0	+92.0	+0.28	82	−9.6
	III	W	384.0	324.0	45.3	+17.7	+0.05	88	+20.7

* W = wheat; L = supplemented lysine; K = supplemented potassium; M = milk.
† Nitrogen balance index is a function of the efficiency of retention of nitrogen for anabolism.
‡ Potassium was given by mouth once daily separately from the formula.
§ Potassium was added to furnish a total intake of 1 mEq./kg./day.

TABLE III

Summary of Effects of Lysine and Potassium Supplementation of Wheat and Milk-Wheat Mixtures*

Baby No.	Lysine	Supplements Lysine + Potassium	Potassium
A. Wheat and Milk Mixtures (75 calories. Less than 2.1 gm. protein/kg./day)			
1	0	+	0†
	0	0	
2	0	0	0†
3	−		
	0		
4	0		
B. Wheat and Milk Mixtures. (75 to 100 calories. More than 2.1 gm. protein/kg./day)			
5	0		
	+		
6	0		
7	0		
	0		
8	0		
9	+		
10	0		
11	0		
C. Wheat Alone. (75 calories. More than 2.1 gm. protein/kg./day)			
12	0	+	†
16	+	+	+
			+
D. Wheat Alone. (100 calories. 2 gm. protein/kg./day)			
17		0‡	
		0‡	
18	0	+	0
			0
19	0	+	0
		+	
20		0‡	
		+‡	
21		+‡	
E. Wheat Alone. (100 calories. More than 2.1 gm. protein/kg./day)			
13		0†‡	†
		0†‡	†
14	0		0†
15		0†‡	†
22		+	+

* A positive (+) effect of the supplement is considered to exist if the nitrogen balance during the supplemented period differs by more than 10 per cent of the nitrogen intake from the most positive control period, a negative (−) effect if the control period exceeds the supplemented period by more than 10 per cent of the intake, and no effect (0) if the balances are between these limits.

† Potassium added once daily. In all others, potassium mixed with feeding.

‡ Control period is wheat and potassium.

The potassium content of wheat and milk was also determined in the same manner.

Stools were dried with concentrated hydrochloric acid, acetone, and heat, and were mixed and pulverized before chemical analysis. For potassium determination both wheat and dry stool were digested with concentrated sulfuric acid and heated until the mixture was clear.

Urine was measured for volume, specific gravity, and pH. Chemical analysis was made for total nitrogen by the micro-Kjeldahl technic, urea by the urease method, ammonia by aeration, uric acid by direct colorimetric method, creatinine by the Jaffe reaction,[18] and amino acid nitrogen by the method of Troll and Cannon.[19]

Serum determinations were made of total protein, albumin, nonprotein nitrogen, cholesterol,[20] urea nitrogen,[21] alpha-amino nitrogen,[19] cholinesterase,[22] sodium, potassium, chloride,[17] hematocrit, and hemoglobin.

Results

The balance data are calculated from the last six days of each period§ and are presented in detail in Table II and summarized as to the effectiveness of supplementation in Table III. In Table III, an effect of a supplement is considered to be present, either positive or negative, when the difference between the best control period and the balance period under

§ Prior to initiating the study, it was planned to interpret the results on the basis of the findings omitting the first three days of each nine-day study. This was performed in order to obtain results more truly reflective of the regimen than to carry over effects and metabolic alterations associated with adjustments to a new feeding regimen. Analysis of the data obtained in the three-day periods indicated a greater variability of the first period with the second and third periods, than the second and third periods with the average of the three periods. Sometimes the first period indicated lesser retention, sometimes greater retention, but more commonly similar retentions as the average.

study differ by more than 10 per cent of the nitrogen intake for the period.

Table IIIA indicates the effect of supplements in four babies (No. 1, 2, 3, and 4, Table II). The basic diet of these four babies was a milk-wheat mixture in which 70 per cent of the protein was obtained from wheat and 30 per cent from milk. The babies were fed 75 calories/kg./day, and the protein content of the diet was less than 2.1 gm./kg./day. In six periods in which the wheat-milk mixture was supplemented with lysine, five balance determinations indicated no effect of the supplementation; and in one period, the lysine-supplemented period produced a negative balance compared with the control period. In two periods in which the wheat-milk mixture was supplemented with potassium (the potassium being given once daily) no effect of the supplementation was noted. In three periods in which the wheat-milk mixture was supplemented with both lysine and potassium, one showed a positive effect, and two no effect.

Table IIIB lists seven babies (No. 5 through 11, Table II) fed a similar mixture except that the caloric intake was 75 to 100/kg. and the protein intake was greater than 2.1 gm./kg./day.

In the nine lysine-supplemented periods, two babies showed an increased retention when the wheat-milk mixture was supplemented with lysine, and seven showed no effect of the supplementation. Of the two infants who showed an increased retention, one (No. 5) received 75 calories/kg./day, and the other (No. 9) 100 calories/kg./day.

Table IIIC summarizes the findings in two babies (No. 12 and 16, Table II), fed wheat alone, 75 calories/kg., and less than 2.1 gm. protein/kg./day.

In the two periods supplemented with lysine alone, one baby showed increased nitrogen retention, and one no effect. When wheat was supplemented with potassium alone there was an increased retention in all three periods studied. When wheat was supplemented with both lysine and potassium there was increased retention in two of the three periods, and no effect in one period. In one baby (No. 16), the the effect of the double supplementation was very large.

Table IIID gives the data for five babies (No. 17 through 21, Table II) fed wheat alone at 100 calories and 2 gm. protein/kg./day. In three babies (No. 17, 20, and 21), the control period was wheat supplemented with potassium. When wheat was supplemented with lysine alone there was no effect in the two periods studied. When wheat was supplemented with potassium alone there was no effect in the three periods studied. A significant effect was noted however, when wheat was supplemented with lysine and potassium. In six of the eight periods studied, there was a positive effect of the supplements, and in two no effect.

Finally, as shown in Table IIIE, wheat alone was fed to another four babies (No. 13, 14, 15, and 22, Table II) at 100 calories/kg., but the protein intake was greater than 2 gm./kg. Three babies (No. 13, 14, and 15) were given their potassium supplement once daily. In two (No. 13 and 15), the control period diet was wheat plus potassium. In the five periods supplemented either with lysine alone, potassium alone, or lysine and potassium no effect was noted. In only one baby (No. 22), who received the potassium mixed with the formula, was there a positive effect on nitrogen retention after potassium supplementation, and an even more marked effect after the addition of lysine and potassium.

Potassium balances were determined in fourteen babies and the balance data are tabulated in Table IV. The results of these balance studies indicate the relationship of increased potassium retention with increased nitrogen retention. Despite the very low potassium intake during potassium-unsupplemented periods, 0.50 to 0.75 mEq./kg./day, the results of only four of eighteen balance studies performed in eight subjects were negative.

Urinary Nitrogen Fractionation (Table V)

The component parts of total urinary nitrogen excretion were analyzed: urea, ammonia, α-amino nitrogen, uric acid, and creatinine. The excretion of urea appeared to correlate closely to the total nitrogen excretion. In the period in which positive effects of supplementation with potassium and lysine were noted,

TABLE IV

Relationship of Potassium Intake and Potassium Balance to Nitrogen Balance and Nitrogen Absorption

Baby No.	Period	Diet	Potassium (mEq./kg./day)				Nitrogen Balance (mg./kg./day)	Per Cent of Nitrogen Absorbed
			Intake	Urine	Stool	Balance		
1	I	WL	0.55	0.254	0.072	+0.223	−11.9	85
	II	W	0.55	0.296	0.160	+0.092	+5.0	84
	III	WL	0.55	0.314	0.159	+0.074	+6.9	86
	IV	WK	1.46	0.935	0.218	+0.310	+13.8	85
	V	WLK	1.44	0.888	0.268	+0.282	+20.0	85
	VI	WLK	1.46	0.935	0.240	+0.287	+24.0	87
2	I	W	0.53	0.240	0.229	+0.060	−15.4	80
	II	WL	0.54	0.262	0.246	+0.030	−10.3	79
	III	W	0.55	0.151	0.210	+0.187	+7.5	82
	IV	WLK	1.44	0.948	0.308	+0.179	+22.4	84
	V	WK	1.44	0.917	0.367	+0.151	+14.4	85
3	I	WL	0.74	0.478	0.238	+0.023	−26.4	88
	II	W	0.74	0.275	0.139	+0.323	+25.4	88
	III	WL	0.74	0.402	0.225	+0.108	+32.8	89
	IV	W	0.74	0.385	0.272	+0.085	+5.4	86
4	I	W	0.76	0.345	0.131	+0.282	−5.8	80
	II	WL	0.75	0.674	0.162	−0.085	−3.4	82
	III	W	0.76	0.556	0.120	+0.082	+12.8	85
5	I	WL	1.17	0.912	0.266	−0.010	+6.2	89
	II	W	1.17	0.824	0.240	+0.105	0	91
	III	WL	1.17	0.792	0.202	+0.177	+43.0	91
6	I	W	1.09	0.533	0.278	+0.280	+14.7	86
	II	WL	1.08	0.700	0.228	+0.154	+28.7	89
	III	W	1.07	0.705	0.189	+0.172	+34.4	91
	IV	M	2.18	1.740	0.099	+0.338	+67.4	93
	V	W	1.07	0.468	0.303	+0.295	+68.3	83
12	I	WL	0.50	0.170	0.113	+0.218	+27.5	90
	II	W	0.50	0.469	0.140	−0.108	+34.5	90
	III	WLK	1.91	1.050	0.421	+0.436	+77.0	97
	IV	WK	1.92	1.270	0.515	+0.126	+64.9	97
	V	WLK	2.00	0.948	0.539	+0.513	+39.4	98
16	I	WK	2.08	0.966	0.912	+0.210	+71.7	74
	II	WLK	3.04	1.865	0.940	+0.235	+130.5	81
	III	WK	3.05	1.820	0.858	+0.372	+104.5	77
	IV	WL	0.54	0.281	0.348	−0.095	+40.5	65
	V	W	0.54	0.184	0.534	−0.174	−10.0	54
17	I	WLK	2.94	2.22	0.720	0	+142.2	92
	II	WK	2.88	2.09	0.596	+0.195	+114.4	92
	III	WLK	2.83	1.83	0.756	+0.244	+95.0	90
18	I	WK	2.96	1.835	0.564	+0.561	+67.3	88
	II	WLK	2.90	2.220	0.453	+0.227	+83.3	85
	III	WK	2.81	1.905	0.355	+0.550	+43.4	88
	IV	WL	0.895	0.436	0.253	+0.206	+49.1	88
	V	W	0.873	0.260	0.217	+0.396	+47.0	88
19	I	WL	0.981	0.332	0.449	+0.200	+41.0	77
	II	W	0.977	0.725	0.612	−0.360	+24.0	75
	III	WLK	2.87	1.638	0.666	+0.566	+59.0	80
	IV	WK	2.84	1.790	0.675	+0.375	+42.0	84
	V	WLK	2.80	1.830	0.560	+0.410	+90.0	86

TABLE IV *Continued*

Baby No.	Period	Diet	Potassium (mEq./kg./day)				Nitrogen Balance (mg./kg./day)	Per Cent of Nitrogen Absorbed
			Intake	Urine	Stool	Balance		
20	I	WLK	3.080	1.270	0.474	+1.236	+81.0	84
	II	M	2.560	1.510	0.231	+0.819	+73.4	88
	III	WLK	2.960	1.880	0.606	+0.474	+70.8	86
	IV	M	2.470	1.390	0.316	+0.764	+78.2	87
	V	WK	2.860	1.272	0.640	+0.948	+40.9	88
21	I	M	2.170	1.540	0.464	+0.166	+77.2	92
	II	WLK	2.960	1.912	0.527	+0.521	+56.5	88
	III	M	2.080	1.480	0.333	+0.267	+86.5	92
	IV	WK	2.840	1.940	0.306	+0.596	+20.5	87
22	I	WK	3.00	1.440	0.820	+0.740	+67.5	86
	II	WLK	2.95	1.440	1.155	+0.355	+92.0	82
	III	W	0.736	0.365	0.147	+0.224	+17.7	88

urinary nitrogen decreased. The component most affected was urea nitrogen. No consistent pattern was noted in the ammonia excretion except that it was found to be less during the milk-feeding periods (babies No. 10, 11, 13, and 17). Likewise, supplementation did not affect the proportion of the urea and ammonia to the total nitrogen.

There was little difference in the alpha-amino nitrogen excretion during the supplemented and unsupplemented periods. In all babies, alpha-amino nitrogen was less than 10 per cent of the total urinary nitrogen.

The uric acid excretion remained constant for each baby and did not vary either with changes in balances or supplementation of the diet with lysine and/or potassium.

Creatinine excretion varied slightly for each baby, but variations were not consistent with regard to nitrogen excretion, balances, or lysine supplementation.

Serum Measurements

Total serum protein, albumin, globulin, and urea nitrogen levels varied little and were within normal limits during the duration of the study of each baby. Cholesterol levels were in the lower range in all the babies throughout the study.

Serum cholinesterase was measured in an attempt to evaluate the status of subjects at the beginning of the study[23,24] and possibly the biologic value of protein. It was found to remain normal during the duration of the experiment, and was unrelated to nitrogen balance.

The serum α-amino nitrogen has been said to serve as an index of the value of dietary protein.[25] The results of the present study showed no relation of variations in serum α-amino nitrogen to changes in nitrogen balance.

The hemoglobin and hematocrit levels were normal and no significant changes were noted during the study. Likewise, serum sodium, potassium, and chloride levels were quite constant during the duration of the study for each baby regardless of the difference in potassium intake.

COMMENTS

The present data indicate that infants remained in nitrogen equilibrium when wheat was supplemented so that 30 per cent of the total protein fed was derived from milk, even when the total protein intake was less than 2 gm./kg./day and the caloric intake was reduced to 75 calories/kg./day. Positive nitrogen balances were obtained when wheat was fed alone or when wheat was supplemented with milk fed at total protein levels of 2 to 4 gm./kg./day. Wheat supplemented with lysine and potassium appeared to be an adequate source of protein in growing infants.

During the early period of the study, eleven babies were fed wheat supplemented with

TABLE V
Urinary Nitrogen Partition (mg. nitrogen/day)

Baby No.	Period	Diet	Total Nitrogen	Urea	Ammonia	Urea + NH_3 / Total Nitrogen	Amino Acid	Uric Acid	Creatinine
1	I	WL	1,013	800	110	0.90	58	37	27
	II	W	900	770	110	0.98	42	36	28
	III	WL	912	720	100	0.90	36	36	26
	IV	WK	863	690	130	9.95	47	35	24
	V	WLK	857	540	140	0.79	50	36	24
	VI	WLK	858	590	110	0.81	67	35	25
2	I	W	1,150	900	130	0.90	57	46	28
	II	WL	1,100	730	130	0.78	77	45	29
	III	W	1,017	820	120	0.98	67	38	28
	IV	WLK	990	650	120	0.78	95	40	27
	V	WK	1,062	690	140	0.78	83	41	28
3	I	WL	1,827	1,270	150	0.78	65	44	23
	II	W	1,483	1,170	120	0.87	85	46	26
	III	WL	1,427	1,210	120	0.93	56	42	27
	IV	W	1,565	1,280	90	0.87	65	42	24
4	I	W	1,290	930	170	0.75	87	47	27
	II	WL	1,308	1,050	130	0.90	91	43	26
	III	W	1,250	1,070	110	0.94	75	44	24
5	I	WL	1,775	1,330	110	0.81	12	33	17
	II	W	1,837	1,550	100	0.87	48	33	21
	III	WL	1,653	1,470	100	0.95	40	31	20
6	I	W	1,852	1,530	110	0.89	45	39	25
	II	WL	1,825	1,550	150	0.93	51	39	24
	III	W	1,852	1,590	110	0.92	51	40	24
	IV	M	1,930	1,820	80	0.98	58	36	24
	V	W	1,880	1,660	150	0.96	48	39	25
7	I	WL	2,600	1,930	122	0.79	45	..	23
	II	W	2,588	2,170	108	0.88	105	..	23
	III	W	2,848	2,410	138	0.89	47	..	27
	IV	WL	3,265	2,350	161	0.77	69	..	27
8	I	W	2,108	1,540	122	0.79	57	..	21
	II	WL	2,158	1,590	116	0.79	47	..	21
	III	W	2,085	1,600	106	0.81	36	..	20
9	I	W	3,067	2,350	150	0.81	67	45	27
	II	WL	2,670	2,070	170	0.84	87	49	27
	III	W	3,133	2,410	150	0.82	70	55	30
10	I	W	2,772	2,170	180	0.81	94	44	24
	II	WL	2,900	2,240	180	0.84	103	40	22
	III	W	2,832	2,080	170	0.78	81	41	22
	IV	W	2,980	2,390	120	0.84	94	41	25
	V	ML	2,813	2,240	120	0.84	95	39	23
11	I	W	2,617	2,050	157	0.85	89	42	24
	II	W	2,502	2,040	150	0.87	91	41	22
	III	W	2,593	1,890	150	0.79	91	42	22
	IV	WL	2,552	1,810	165	0.77	89	41	21
	V	W	2,568	2,030	143	0.85	92	40	20

TABLE V *Continued*

Baby No.	Period	Diet	Total Nitrogen	Urea	Ammonia	Urea + NH_3 / Total Nitrogen	Amino Acid	Uric Acid	Creatinine
12	I	WL	1,790	1,060	198	0.70	105	47	29
	II	W	1,735	1,360	167	0.88	82	49	30
	III	WLK	1,520	1,240	159	0.92	49	48	27
	IV	WK	1,605	1,390	162	0.96	66	49	28
	V	WLK	1,783	1,360	155	0.85	78	47	24
13	I	WLK	1,825	1,270	134	0.77	47	..	24
	II	WK	2,103	1,780	95	0.89	42	..	29
	III	WLK	2,328	1,760	129	0.81	34	..	27
14	I	W	1,893	1,050	220	0.67	82	..	21
	II	WL	1,757	890	243	0.64	90	..	18
	III	WK	1,823	1,050	165	0.67	77	..	17
15	I	WK	1,537	1,140	162	0.84	51	..	15
	II	WLK	1,550	1,140	178	0.84	48	..	15
	III	WK	1,665	1,190	196	0.83	46	..	16
16	I	WK	1,232	990	135	0.91	39	49	26
	II	WLK	945	812	118	0.98	59	48	26
	III	WK	1,070	952	102	0.98	38	48	27
	IV	WL	1,230	958	160	0.91	78	48	27
	V	W	1,365	1,040	94	0.84	61	49	28
17	I	WL	963	785	146	0.96	81	41	26
	II	W	1,137	898	111	0.89	76	47	27
	III	WL	1,240	837	154	0.80	124	44	28
18	I	WK	1,475	1,132	169	0.88	106	43	24
	II	WLK	1,270	703	143	0.67	77	43	27
	III	WK	1,615	1,178	162	0.83	87	46	31
	IV	WL	1,567	943	197	0.73	113	45	29
	V	W	1,575	1,208	187	0.89	85	46	30
19	I	WL	1,350	792	257	0.78	150	46	30
	II	W	1,425	907	220	0.79	126	48	29
	III	WLK	1,275	810	195	0.79	127	53	29
	IV	WK	1,477	1,135	177	0.89	109	56	28
	V	WLK	1,133	878	172	0.93	126	62	26
20	I	WLK	1,047	642	152	0.76	64	38	25
	II	M	950	663	85	0.79	66	42	27
	III	WLK	1,007	733	138	0.77	65	39	26
	IV	M	890	770	107	0.98	79	39	27
	V	WK	1,200	863	110	0.81	74	45	28
21	I	M	1,312	1,083	105	0.91	58	42	32
	II	WLK	1,098	763	174	0.85	62	39	29
	III	M	1,227	890	113	0.82	51	39	34
	IV	WK	1,312	1,060	118	0.90	86	37	32
22	I	WK	1,503	1,232	181	0.94	55	36	24
	II	WLK	1,283	1,035	187	0.95	101	43	23
	III	W	1,822	1,483	170	0.91	107	47	21

milk with 30 to 40 per cent of total protein derived from milk. The protein intake varied from 1.2 to 3.5 gm./kg./day and 75 to 100 calories/kg./day. Only two babies (No. 5 and 9) showed a beneficial effect of lysine. The milk protein itself may have been adequate to correct the lysine deficiency in wheat for these infants.

In eleven babies, wheat alone was the source

of protein. In four, the supplemented potassium was given separately from the formula. No beneficial effect of lysine or potassium was demonstrated.

Improvement in value of wheat protein may be obtained when supplemented with lysine in an amount calculated to give the wheat a total content of 0.55 per cent lysine when adequate potassium is present. In seven babies potassium was mixed with the formula. In these babies (No. 16 through 22) the effect of the lysine in increasing nitrogen retention was demonstrated in all except one (No. 7) in whom nitrogen retention was remarkably high throughout the study.

Two babies showed an efficiency of protein for anabolism close to milk protein. The observed effect of lysine is apparently an improvement in the efficiency of retention of nitrogen for anabolism as no effect on nitrogen absorption was observed.

Simultaneous availability of potassium for maximal utilization of protein appears to be an important factor. It is generally accepted that nitrogen retention can be increased if protein is given simultaneously with potassium.[26–28] Improving protein quality, therefore, might not increase nitrogen retention unless accompanied by a simultaneous increase in potassium intake or any other nutrient which is marginal to the diet.

In the present study, one baby (No. 3) retained less nitrogen when lysine was added to wheat than in the lysine-unsupplemented periods. Eckel and Norris[14,29] demonstrated that increasing lysine accentuated potassium deficiency. In muscle cells, lysine replaced the cationic effects of potassium intracellularly without apparent loss of known cellular functions. Muntwyler and co-workers[30] found, in rats fed a diet deficient in potassium, that there was a loss of skeletal potassium, which was replaced by sodium. They found that the normal intracellular cation in the rat was potassium, probably associated with glutamic and aspartic acids. On potassium depletion, the basic substances of the cell were lysine and arginine. Gershoff et al.[31] found that in rats potassium could not substitute for lysine in a lysine-deficient diet.

In technically underdeveloped countries where vegetables constitute the chief source of proteins, and where kwashiorkor develops in children because of protein insufficiency, the children often have potassium insufficiency as well.[32] It is easy to assume that the potassium deficiency is due to chronic loss in the feces. The practice of feeding thin gruels deficient in all nutrients serves to aggravate pre-existing deficits.

The present study indicates that potassium increases the protein absorption and retention of highly refined wheat. Experience as obtained herein suggests that the potassium requirement may increase when the quality of refined wheat protein is improved by the addition of lysine, and confirms that a lysine-potassium interrelation is present in man.

Thus, in areas in which total protein intake from vegetables is marginal, improvement in the quality of protein may follow the addition of the limiting essential amino acids, provided there is adequate native potassium present or supplementary potassium is mixed with the diet.

Lysine requirements for minimal maintenance of adults are relatively well established at about 0.4 gm. per day on semi-synthetic diets.[33–36] The lysine requirement for the growing infant, however, is not well established. Albanese,[37] using carcass analysis, has estimated this requirement as 170 mg./kg./day. Snyderman et al.,[38] using balance technics, have estimated the lysine requirement for the infant at 90 mg./kg./day. By supplementing the wheat diet in the present study to bring the lysine closer to the FAO pattern, the amount of lysine actually fed the infants who received 2 gm. of wheat protein/kg. and who remained in positive balance is 85 to 90 mg./kg./day. This suggests that the requirement of essential amino acids, with special regard to lysine, may need modification to indicate that the amino acid requirement should be stated in terms of total protein in the diet and of proportions of one to another for the maximal utilization of protein. Thus, in low protein diets, a lower quantity of an essential amino acid may be necessary than in high protein diets.

Even further, a probably optimal relationship exists between total caloric intake and total protein intake. Very little experimentation has been carried out to determine this factor in man. In rats, Yoshida, Elevehjem and Harper[39] have determined that optimal protein utilization occurs when the diet consists of 60 mg. of protein per calorie. Forbes, Vaughan and Yohe[40] have shown that with adequate calories, the biologic value of protein decreases linearly as the protein content of the diet increases.

Other deficiencies may exist in wheat diets but were not revealed by these studies. All these babies were given vitamin supplements, and the period of study may have been too short to reveal other deficiencies such as those due to fats and fatty acids.

SUMMARY

In order to evaluate lysine as a supplement to wheat to improve nitrogen retention of infants fed white wheat flour as a chief source of protein, twenty-two male malnourished infants were studied by nitrogen balance technics. Infants were fed 1.2 to 4 gm. of protein/kg./day and 75 to 120 calories/kg./day. Most consistent effects of supplementation were obtained at protein intakes of 1.75 to 3 gm./kg., caloric intakes of 75 to 100 calories/kg./day, and over 2 mEq. potassium/kg./day.

The nutritional value of refined wheat protein can be improved by simultaneous supplementation of lysine and potassium. The effect of lysine was demonstrated confirming previous animal experiments. The data also indicate the essentiality of an adequate potassium intake for efficient protein metabolism.

The data indicate that the value of refined wheat protein supplemented with potassium and lysine is close to milk. Wheat supplemented with potassium and lysine, as determined in this study, is an adequate source of protein for growing infants for the periods covered by this study. Wheat-milk mixtures were not improved by supplementation with lysine and potassium. The lysine and potassium were effective only when mixed with the daily wheat diet.

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Renal Clearance of Calcium and Phosphorus in Experimental Urolithiasis

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THE occurrence of urolithiasis has long been a clinical problem in man as well as almost every other species in which it has been studied. The frequent presence of stones and/or calcification in kidneys of ulcer patients maintained on milk and alkali diets led to the description of a "milk-alkali syndrome" by Burnett et al.[1] in 1949. Since then, many references to specific cases, such as those included in a review by Kushner,[2] testify to the concern about urolithiasis in man.

So many factors have been implicated in urolithiasis that the etiology remains obscure. Several of the factors which have been suggested are mineral imbalance, avitaminosis A, hypercalcemia, hypercalciuria, hyper- and hypophosphatemia, hypervitaminosis D, hyperparathyroidism, infection, low pH, lack of colloids in urine, alkalosis, endocrine disturbances, skeletal neoplasms, dehydration, dietary deficiencies and excesses. It is clear that many of these are interrelated so that the over-all picture is complex.

For that reason several approaches to studying urolithiasis under experimental conditions have been initiated in recent years. Yarbro[3–7] in a series of studies on human beings investigated the physiochemical relationships of calcium in the urine of habitual calculus formers. Sager and Spargo[8] produced urinary calculi in protein-depleted rats on a low phosphorus diet, and by means of balance studies found that renal excretion of calcium and citrate were increased, precipitating as calcium citrate calculi. In their study on guinea pigs, when Maynard et al.[9] produced calcification in the kidney by creating a mineral imbalance (lowered magnesium in the diet), they found serum magnesium was lowered and serum phosphorus was increased. No report was found in the literature in which an attempt was made to study the functional activities of calcified kidneys as far as filtering and reabsorbing certain blood constituents such as calcium and phosphorus were concerned. That renal function is altered in the presence of urolithiasis was shown by Bjorneboe et al.,[10] who found that it was only 50 per cent of normal in two patients with calcinosis renis.

It appeared that a study of renal clearances in the presence of urolithiasis would be valuable in the understanding of basic processes leading to calcification. Since the rat has been firmly established as a valuable experimental animal in which to study human afflictions and since methods of making renal clearance studies in this animal were available in the literature, the rat was chosen for the present study. Urolithiasis was known to be producible by the feeding of a milk-alkali diet devised by Sambhavapol et al.[11] and modified by Empey.[12] Knowing that it could be produced by this dietary means, it was possible to study renal functions in both rats with urolithiasis and healthy ones by measuring the filtration and reabsorption of calcium and phosphorus in the two groups of animals.

METHODS AND PROCEDURES

Fifty albino female weanling rats were used. Thirty were of the Yale strain which has

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been maintained as a stock colony at the Cornell small animal laboratory for about thirty years; the others were of the Wistar strain. Ten of each strain were given a "non-calculus-producing" diet of ground Big Red Dog Food and ten of each were fed the following "calculus-producing" diet used by Empey.[12]

Parlac (dry whole milk)	1,000 gm.
$NaHCO_3$	30 gm.
Solka floc (cellulose)	20 gm.
Delsterol (vitamin D_3)	0.66 gm.
$MnCl_2.4H_2O$	115 mg.
$CuSO_4.5H_2O$	62 mg.
$FeCl_2.6H_2O$	420 mg.
KI	160 mg.

The ten remaining Yale strain rats were given the same milk-alkali diet plus 10 mg. per cent of magnesium. The trace minerals were added at levels to prevent known mineral deficiencies. All diets were fed *ad libitum* and distilled water was freely accessible.

The animals were maintained on the respective diets from the time of weaning until nine months of age, at which time each animal was subjected to a renal clearance test. Empey[12] had found that kidney calcification was detectable at seven months in female rats on the milk-alkali diet.

Within ten days of the time each renal clearance study was performed, each animal was unilaterally nephrectomized so that a roentgenogram of one kidney could be taken. The degree of calcification was determined by an arbitrary rating of the roentgenogram, on a scale from 1 to 6, with 1 representing no calcification and 6 representing massive calcification.

The animals were then allowed to recover approximately one week, after which time they were given a diet they had not received previously. Those which had been receiving the two milk-alkali diets were given laboratory chow and those which had been receiving chow were given the milk-alkali diet without the magnesium supplement. This time the laboratory chow was supplemented with 1 per cent ammonium chloride in an attempt to reverse calcification. After periods of from thirty-five to sixty-nine days on these diets, the rats were sacrificed, the remaining kidney was removed, a roentgenogram was taken and rated as the previously excised organ had been.

Modified methods of Friedman et al.[13] and Corcoran et al.[14] for renal clearance procedures, using *p*-aminohippurate to measure renal plasma flow, were used. The filtration and reabsorption rates of calcium and phosphorus were determined by measuring the respective elements in plasma and urine. Calcium was analyzed by the method of Bachra et al.[15] and inorganic phosphate was determined by modifications of the method of Shinowara et al.[16] *P*-aminohippurate concentrations were measured by the method described by Friedman et al.[13] The necessary modifications of these methods were set forth in detail by Payne.[17]

Statistical interpretation of data incorporated the methods of Snedecor.[18]

RESULTS AND COMMENTS

In all animals on the milk-alkali diets, an 85 per cent incidence of calcification occurred in comparison with an incidence of 20 per cent among animals on the control diet of ground laboratory chow. The Wistar strain rats showed a little more susceptibility to urolithiasis on the control diet and a greater degree of calcification on the milk-alkali diets.

The means of degree of calcification of the two groups of Yale strain rats receiving the milk-alkali diet and the milk-alkali diet plus magnesium were 2.3 ± 0.9 and 2.3 ± 1, respectively, as compared to 1.1 ± 0.4 in control animals. Calculation of the analysis of variance between each group and the control group resulted in "P" values of 0.14 and 0.20, respectively. In the rats of Wistar strain, the mean degree of calcification was 2.9 ± 1 in the group receiving the milk-alkali diet and 1.3 ± 0.5 in the control group. The "P" value in this case was 0.20. Although the analyses of variance did not show a statistically significant difference at the 5 per cent level between control animals and afflicted animals, the "P" values did indicate there was a biologic

TABLE 1

Filtration and Reabsorption of Calcium and Phosphorus in the Kidneys of Yale Strain rats With and Without Renal Calcification

Rat No.	Effective Renal Plasma Flow (ml./min.)	Glomerular Filtration Rate (ml./min.)	Urine Flow (ml./min.)	Calcium				Phosphorus			
				Filtered (μg./min.)	Excreted (μg./min.)	Reabsorbed (μg./min.)	Per Cent Reabsorbed	Filtered (μg./min.)	Excreted (μg./min.)	Reabsorbed (μg./min.)	Per Cent Reabsorbed
				Diet I							
1	2.73	0.428	0.0289	27.0	0.6	26.4	97.8	30.4	4.7	25.7	84.5
3	3.63	0.571	0.0267	38.4	0.0	38.4	100.0	43.4	4.8	38.6	88.9
4	2.84	0.445	0.0311	28.0	6.1	21.9	78.2	91.7	2.4	89.3	97.4
5	0.96	0.149	0.0133	9.0	0.4	8.6	95.6	6.7	0.5	6.2	92.5
6	1.25	0.198	0.0200	5.3	0.4	4.9	92.5	12.7	0.5	12.2	96.1
7	2.00	0.315	0.0200	22.9	0.0	22.9	100.0	15.1	0.8	14.3	94.7
8	2.41	0.378	0.0211	17.5	1.1	16.4	93.7	30.6	0.6	30.0	98.0
9	1.00	0.157	0.0178	4.3	0.0	4.3	100.0	11.5	0.3	11.2	97.4
10	2.04	0.320	0.0244	20.2	0.0	20.2	100.0	17.9	0.6	17.3	96.6
				Diet II							
12	0.96	0.150	0.0133	10.7	1.7	9.0	84.1	13.5	0.9	12.6	93.3
13	2.51	0.394	0.0233	22.7	2.0	20.7	91.2	68.2	3.6	64.6	94.7
14	3.82	0.600	0.0411	33.8	0.9	32.9	97.3	58.2	4.8	53.4	91.7
15	2.30	0.361	0.0367	21.4	2.2	19.2	89.7	21.3	0.8	20.5	96.2
16	2.92	0.457	0.0244	27.1	6.1	21.0	77.5	26.5	0.7	25.8	97.4
17	1.66	0.261	0.0156	14.1	4.5	9.6	68.1	20.4	0.7	19.7	96.6
18	2.03	0.317	0.0300	11.2	1.4	9.8	87.5	28.5	2.3	26.2	91.9
				Diet III							
21	1.45	0.225	0.0200	8.1	0.0	8.1	100.0	32.9	3.3	29.6	90.0
23	2.35	0.367	0.0289	8.1	0.0	8.1	100.0	34.9	1.4	33.5	96.0
24	0.80	0.125	0.0133	5.6	0.0	5.6	100.0	19.1	2.1	17.0	89.0
25	4.04	0.634	0.0289	31.6	3.4	28.2	89.2	54.4	1.3	53.2	97.6
26	1.81	0.282	0.0289	14.2	0.4	13.8	97.2	30.2	0.6	29.6	98.0
27	1.58	0.248	0.0167	12.9	3.0	9.9	76.7	22.1	1.4	20.7	93.7
28	2.06	0.326	0.0267	23.3	5.9	17.4	74.7	28.0	0.9	27.1	96.8
29	0.78	0.123	0.0133	6.9	0.7	6.2	89.9	15.3	0.6	14.7	96.1
30	2.95	0.461	0.0289	31.0	2.9	28.1	90.6	88.5	7.8	80.7	91.2

significance in the occurrence of urolithiasis in animals which received the milk-alkali diets. The significance of 5 per cent usually sought in biologic data might have been obtained had the experimental period been longer. The present experiment was not designed to test the idea.

The difference in mean values for calcium in plasma as between strains and between healthy and afflicted animals was not significant. The average values were in a normal range from 7.9 to 9.5 mg. per 100 ml. Thus, in the present study, the hypercalcemia implicated by other investigators in urolithiasis did not appear to play a part.

The mean level of phosphorus in plasma was significantly higher in the Yale strain rats receiving the milk-alkali diet plus magnesium (12.0 ± 4.4 mg. per 100 ml.) than in control animals (8 ± 3.3 mg. per 100 ml.). The "P" value was 0.04. Although the level of phosphorus in the plasma of the group receiving the milk-alkali diet without magnesium was not significantly higher (9.2 ± 3.7 mg. per 100 ml.), it was higher than in the control animals. In the Wistar strain rats, the mean level of phosphorus was 7.9 ± 2.8 mg. per 100 ml. plasma in the group receiving the milk-alkali diet and 13.9 ± 5.0 mg. per 100 ml. in the control group. The "P" value in this case was 0.005.

Hyperphosphatemia occurred in Yale rats only when magnesium was added to the milk-alkali diet and in Wistar rats when no supplement of magnesium was given. This seeming

TABLE II

Filtration and Reabsorption of Calcium and Phosphorus in the Kidneys of Wistar Strain Rats With and Without Renal Calcification

Rat No.	Effective Renal Plasma Flow (ml./min.)	Glomerular Filtration Rate (ml./min.)	Urine Flow (ml./min.)	Calcium				Phosphorus			
				Filtered (μg./min.)	Excreted (μg./min.)	Reabsorbed (μg./min.)	Per Cent Reabsorbed	Filtered (μg./min.)	Excreted (μg./min.)	Reabsorbed (μg./min.)	Per Cent Reabsorbed
Diet I											
31	1.63	0.245	0.0158	14.0	0.0	14.0	100.0	20.6	0.4	20.2	98.1
32	2.83	0.444	0.0289	26.4	0.0	26.4	100.0	28.9	1.4	27.5	95.2
33	1.31	0.204	0.0156	12.6	0.0	12.6	100.0	15.8	0.8	15.0	94.9
34	3.98	0.625	0.0195	37.9	5.4	32.5	85.8	31.9	0.6	31.3	98.1
35	3.05	0.480	0.0317	13.2	0.6	12.6	95.5	46.1	1.3	44.8	97.2
36	1.96	0.306	0.0156	15.1	0.3	14.8	98.0	24.5	1.7	22.8	93.1
38	3.96	0.620	0.0267	16.0	5.0	11.0	68 8	67.0	0.9	66.1	98.7
39	1.39	0.219	0.0089	6.3	0.0	6.3	100.0	15.8	1.6	14.2	89.9
40	2.17	0.339	0.0359	20.1	3.3	16.8	83.6	26.1	1.5	24.6	94.3
Diet II											
41	2.48	0.387	0.0222	22.5	12.4	10.1	44.9	42.6	3.7	38.9	91.3
42	3.58	0.564	0.0289	32.5	11.5	21.0	64.6	34.4	4.9	29.5	85.8
43	3.49	0.550	0.0452	18.5	16.1	2.4	13.0	64.4	1.8	62.6	97.2
44	2.19	0.346	0.0267	22.4	9.8	12.6	56.3	65.4	4.5	60.9	93.1
45	2.00	0.314	0.0178	24.5	7.7	16.8	68.6	35.2	3.5	31.7	90.0
46	2.24	0.351	0.0244	18.7	1.0	17.7	94.7	35.5	1.2	34.3	96.6
47	1.73	0.271	0.0200	17.6	1.5	16.1	91.5	58.5	3.3	55.2	94.4
48	1.66	0.260	0.0167	10.3	3.7	6.6	64.1	29.6	0.5	29.1	98.3
49	1.00	0.157	0.0144	9.1	2.5	6.6	72.5	37.7	0.5	37.2	98.7
50	2.00	0.314	0.0211	17.3	0.0	17.3	100.0	40.5	3.2	37.3	92.1

contradiction in regard to magnesium might indicate that the levels of calcium and magnesium in plasma are additive and that phosphorus exists in proportion to the total of the two elements rather than just calcium as expressed in the following relationship mentioned by Kushner:[2]

$$\frac{Ca^{++} \times HPO_4^{=} \times HCO_3^{-}}{pH} = K$$

There was no excess of phosphorus or deficiency of calcium in the milk-alkali diet, so that hyperphosphatemia mediated through hyperfunction of the thyroid, caused by hypocalcemia, did not appear to offer an explanation. The ratio of calcium to phosphorus was 1.3:1 in the milk diet, which lay within the range of ratios of 2:1 to 1:1 considered to be favorable for growth and bone formation. Only the fact that phosphorus was completely reabsorbed after filtration in the kidney, whereas calcium was not completely reabsorbed as in normal kidneys, seemed to explain the hyperphosphatemia in the present study.

The results of the renal filtration and reabsorption of calcium and phosphorus are shown in Tables I and II. The effective renal plasma flow values, as determined by measuring renal clearances of *p*-aminohippurate, were lower than values summarized by Smith.[19] However, our values were for female rats which, of necessity, were lightly anesthetized immediately before the collection period and analyses were made on blood taken by heart puncture. The fact that both control animals and afflicted animals were subjected to a highly standardized procedure appeared to validate the results for the comparative purposes we were interested in.

The glomerular filtration rate was calculated by multiplying the effective renal plasma flow per 100 gm. body weight by a factor of 0.16 on the basis of Smith's[19] figure of 0.157 ± 0.023 calculated from Friedman's[13] data. It was realized that it would be more satisfactory

to actually measure glomerular filtration rate in each individual animal, but so many analyses were being made on approximately 1 cc. plasma and 1 cc. urine that another analysis seemed prohibitive. Since the literature indicated the 0.157 was fairly constant, even among investigators, it was considered valid to apply the factor as long as interpretation of results were made in light of that application.

The quantity of calcium filtered was calculated by multiplying the calcium in the plasma by a factor of 0.6, a compromise figure between the 50 per cent and 75 per cent figures found in the literature for the diffusible fraction of calcium. Phosphorus was considered to be 100 per cent diffusible.

Data in Tables I and II show that calcium reabsorption was inhibited. The possibility existed that the actual glomerular filtration rate was impaired by calcification. However, the greater quantities of calcium excreted by the afflicted rats contraindicated the impairment of filtration, but indicated rather the inhibition of reabsorption. In only one instance was the rate of calcium reabsorption above 21 μg per minute in an animal (No. 25) with definitely calcified kidneys in contrast to rates up to 38.4 μg. per minute in healthy animals. The filtration and reabsorption of phosphorus, on the other hand, remained the same in healthy and unhealthy animals. It would appear, therefore, that the tubular reabsorption of calcium was independent of phosphorus, as stated by Fanconi,[20] and that the apparent imbalance of the two elements in plasma was a reflection of the kidney's handling of them.

Another question arises as to the role that sodium bicarbonate plays in the process of urolithiasis. Although calcification of the kidney occurs frequently in aged animals without sodium bicarbonate appearing in the diet as such, the presence of kidney stones in the older animal may reflect an accumulative effect of this or any other ion. The bicarbonate ion was included in the formula presented by Kushner[2] for the relationships between calcium and phosphorus in plasma. If the bicarbonate was being filtered out at the kidney with no reabsorption, then the presence of calcium (not being reabsorbed) and the absence of the phosphate (due to complete reabsorption) would be conducive to precipitation in the urinary tract. Unfortunately, the calculus material was not analyzed, nor were renal clearances of bicarbonate obtained. Future work on both these phases is anticipated.

Comparisons of the degree of calcification between the right kidney (removed surgically at time of renal clearance tests) and the left kidney (removed at death thirty-five to sixty-nine days later) showed no definite change. The animals which had been changed from the milk alkali diet to laboratory chow plus 1 per cent ammonium chloride in the interim did not show any evidence of reversal of calcification. Nor was there any indication of the development of calcification in those animals changed from laboratory chow to the milk-alkali diet during the intervening period. Unfortunately, only an average of five of every group of ten survived to be changed from one dietary regimen to another. The group III Yale strain rats were sacrificed immediately after renal clearance tests were performed.

It would appear that more studies of this type would not only unravel some of the complexities of urolithiasis, but also shed light on some basic facts about metabolism in general as it is reflected in blood levels of certain constituents which are normally filtered by the kidney. Whether calcification is the cause or the effect of changes in renal function could possibly be answered by such technics. It seems possible that urinary calculi are of different compositions depending upon the particular imbalance that exists in the tubules and urinary tract as a result of the filtration and reabsorption of imbalanced proportions of ions from the blood. If reversal of urolithiasis is to be accomplished, an understanding of such processes would be invaluable.

SUMMARY AND CONCLUSIONS

In a study of the renal filtration and reabsorption of calcium and phosphorus in healthy albino female rats and ones with urolithiasis, it was found that with calculus-producing diets calcium reabsorption was

inhibited, while phosphorus reabsorption was not. Hyperphosphatemia was found in Wistar strain rats receiving a milk-alkali diet and in Yale strain rats receiving the same milk-alkali diet with 10 mg. per cent of magnesium added. Wistar strain rats showed a slightly greater tendency toward urolithiasis than Yale strain rats, but not significantly so. No indication of reversal of calcification was found in animals given laboratory chow plus 1 per cent ammonium chloride, nor was urolithiasis observed in animals given a milk-alkali diet, after the nine-month experimental period.

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Follow-Up Study of Serum Cholesterol, Diet and Physical Findings of Italian-American Factory Workers

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VARIOUS investigators have observed that the incidence of atherosclerosis and coronary artery disease is higher in certain areas of the world than in others. The observations of some of these researchers support the hyperlipophagia-hyperlipemia hypothesis of partial causation in these diseases. Keys and co-workers,[1,2] for example, noted that Neapolitan laborers eat less fat (about 20 per cent of their calories), have lower serum cholesterol levels and have less heart disease than do American laborers whose diet supplies about 40 per cent of their calories from fat. They propose that the advantageous position of the Neapolitan laborers with respect to serum cholesterol level and heart disease is more due to diet than "race," since Neapolitan professional men and Bolognese workers whose diets are richer in fat also have higher levels of serum cholesterol and more coronary artery disease.[2]

In 1956 Miller et al.[3] studied the diet, blood lipids and health of 189 men in Boston who had lived all their adult lives in the United States, but whose parents were born near Naples. They compared the serum cholesterol level and diet of the Neapolitan Bostonians with that of the laborers observed by Keys[1] in Naples. The subjects were between the ages of twenty and fifty years and were employed by the B. F. Goodrich Footwear and Flooring Company in Watertown, Massachusetts. The study[3] indicated that the Bostonians of Italian descent had a mean serum cholesterol level and dietary fat intake similar to other Americans and significantly higher than that of native Neapolitans. In 1958, the participants of the 1956 survey were asked to cooperate in a follow-up study.

METHOD

Complete records were obtained from 147 of the original group of 189. Of the forty-two subjects excluded in the 1958 follow-up study about half (eighteen) were no longer working at the factory; ten were ill, on vacation, or for some other reason were unable to come for their examination as scheduled; and eleven men did not care to participate. Three other subjects were excluded because of incomplete data (the tubes containing serum of two subjects broke and data on smoking were not obtained from the third subject).

The follow-up study included a detailed physical examination, comparable to that given in the first study, and in addition various anthropometric measurements. Laboratory studies included a blood sample for serum cholesterol determination, urine specimen for determination of sugar and albumin, and a twelve-lead electrocardiogram. In 1958, the serum cholesterol level was measured by the micromethod of Carpenter, Gotsis and Hegsted[4] whereas in 1956 the cholesterol level was determined by the method of Abell et al.[5]

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TABLE I
Mean Total Cholesterol Concentration and Mean Relative Weight, with Age

Age (yr.) (in 1956)	No.	Total Cholesterol (mg. %)				Relative Weight			
		1956		1958		1956		1958	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
20-29	25	232	41	222	37	107	12	107	12
30-39	59	238	41	232	44	112	13	112	13
40-49	60	247	46	238	49	107	12	106	12
50+	3	258	37	256	58	106	12	103	5
All ages	147	241	43	233	45	109	13	109	12

The results obtained by the micromethod of Carpenter are in good agreement with those obtained with the standard colorimetric method of Abell.

The relative weights of the subjects were computed, using the tables of standard weights for age, sex and height determined from the Medico-Actuarial Investigation of the Metropolitan Life Insurance Company.[6] This table allows for a moderate increase in weight with age.

Dietary data were collected from a subsample of thirty-nine men (approximately a quarter of the sample) by a Burke-type[7] research dietary interview. This report will be limited to discussing cholesterol levels, weight, smoking and dietary findings.

RESULTS

The means for serum cholesterol levels and relative weight for age groupings according to decades are presented in Table I. The mean serum total cholesterol for the entire sample of 147 subjects was 233 mg. per 100 ml. in 1958. This was slightly lower than the mean of 241 mg. per 100 ml. for this group in 1956. As expected, a slight increase with age was noted. No differences in mean relative weight for the group were observed between the two studies.

The relationship of cigarette smoking to blood cholesterol level and relative weight for this follow-up study is shown in Table II. The subjects were divided into three groups: (1) those who did not smoke, (2) those who smoked less than one package of cigarettes a day, and (3) those who smoked one or more packages a day. The mean serum cholesterol level of the men who smoked one or more packages of cigarettes a day was significantly higher ($p < 0.05$) and their mean relative weight

TABLE II
Mean Total Cholesterol and Relative Weight with Cigarette Smoking

Packs of Cigarettes (per day)	No.	Cholesterol (mg. %)	Relative Weight
None	36	223*	112*
Less than one	42	231	110
One or more	69	241*	106*
All smokers	111	237	108

* Significant ($p < 0.05$)

TABLE III
Mean Total Cholesterol and Factors Associated with Atherosclerosis

No. of Subjects	No. of Factors Possibly Associated with Atherosclerosis*	Mean Serum Cholesterol (mg. %)
8	0	213
35	1	232
69	2	236
25	3	243
2	4	242
Total 139†		

* Family history, obesity, smoking and hypertension.
† Incomplete data on eight subjects.

TABLE IV

Comparison of Two Dietary History Interviews of Thirty-Nine Italian-American Subjects at Two Different Periods

	1956		1958		r†	t‡
	Mean	SEx*	Mean	SEx̄*		
Calories	3,507	132	3,367	127	0.59	N.S.§
Protein (gm.)	133	4.96	135	5.76	0.49	N.S.
Fat (gm.)	164	6.73	163	6.89	0.43	N.S.
Alcohol (gm.)	38	6.41	27	4.80	0.52	Significant
% Calories						
Protein	14.5	0.37	15.4	0.27	0.25	N.S.
Fat	41.0	1.07	43.0	0.95	0.62	N.S.
Alcohol	6.9	1.05	5.6	1.00	0.61	N.S.

* Standard error of the mean.
† Correlation coefficient.
‡ Students paired "t."
§ N.S. = Not significant.

was significantly lower ($p < 0.05$) than that of nonsmokers. The differences between the cholesterol level and relative weight of the other groups were not significant. The lower relative weight of smokers could not be accounted for by the food intake they reported. In fact, when the thirty-nine subjects included in the dietary interview group were separated into two categories, i.e., smokers and nonsmokers, the mean calorie intake was 3,607 for the former and 3,270 for the latter. (The difference was not significant.)

The question of whether obesity plays a role in establishing serum cholesterol level certainly is not elucidated by this study. Although the mean cholesterol level of men with a relative weight of 100 per cent or less was lower (220 mg. per 100 ml.) than that of men whose relative weight was 120 per cent or more (231 mg. per 100 ml.), the difference was not significant. On the other hand, the data in Table II show that heavy smokers have a significantly higher serum cholesterol level but significantly lower relative weight than nonsmokers.

Various investigators are of the opinion that certain factors, namely, heredity, high serum cholesterol levels, obesity, smoking and hypertension have a cumulative effect in causation of coronary arterial and heart disease. We found a trend but no significant differences between the level of serum cholesterol and the number of factors which could be ascribed to the subjects as is shown in Table III. The groups compared, however, were small.

Table IV gives the results of dietary interviews obtained from a subsample of thirty-nine men in 1958 and compares it with data secured from these same persons by the same method in 1956. There were no significant differences except for grams of alcohol. Alcohol consumption may have decreased in 1958 for economic reasons.

No significant dietary changes were observed when the group was considered as a whole, except for grams of alcohol, but considerable individual variations in food intake between the two periods are indicated by the correlation coefficients. In general, the correlation coefficients for the dietary factors were in the neighborhood of 0.4 to 0.6. However, the correlation for the per cent of calories from protein was 0.25, although the mean per cent of calories supplied by protein was approximately 15 for both years (14.6 in 1956 and 15.4 in 1958). This low correlation might be explained in part by the fact that the range for the per cent of calories from protein is narrow; in our sample the range was from 10 to 18.9.

To evaluate the interviews further, the diet records were assessed by a method suggested by Epstein and his associates,[8] whereby (1) the basal caloric requirement is estimated for each person from the Boothby tables,[9] (2) an allow-

ance for activity is added to the basal figure, and (3) the calculated intakes are considered acceptable if they fall within 30 per cent of this figure. About two-thirds (twenty-seven) of the subjects' histories were "acceptable" in both 1956 and 1958. And of the thirty-nine men thirty-two fell into the same category, i.e., "acceptable" or "not acceptable" in both studies. Seven men fell into a different category in 1958 than they had in 1956. In three the intake was reported as excessive in 1956 but in the "acceptable" range in 1958. Of these three, one had lost 13 pounds, one 12 pounds and one showed no change in weight. In the remaining four the intake was in the "acceptable" category in 1956 but reported as excessive in 1958. Three of these men gained weight, 7, 6 and 5 pounds, respectively; the other subject had no change in weight. Although the sample is small, it does indicate that these persons tend to stay in their own category, or if they fall into different categories, the changes can be accounted for, in most cases, by weight gain or loss. Whether or not the use of the Boothby-Berkson[9] chart results in estimates of caloric requirement which are too high is subject to question. The mean percentage of caloric intake in relation to basal metabolic requirement for the 189 subjects interviewed in 1956 was 103 per cent above the basal requirement.[3] This may seem excessively high in view of the activity allowance of 50 per cent calories above the basal requirement for moderately active subjects and 60 per cent above the basal requirement for more rigorous workers used by Epstein et al.[8] However, in a study of Scottish clerks and miners, Garry and co-workers[10] reported an average intake that was 80 per cent above the basal requirement for the former and 147 per cent for the latter.

The results of this small repeat study of the dietary interview indicate that for the group, dietary information collected at one year reflects the general eating pattern for both years. There would have been no need to repeat the dietary interviews to determine the groups' dietary pattern under ordinary conditions.

However, individual data for the two years showed considerable variation. This may be due to various factors: First, the interview method is far from infallible; in fact, it can be grossly inaccurate. It does, however, seem to place groups in several broad classifications. The mean daily caloric intake of a sedentary group of university faculty members was estimated by us to be 2,460 calories, using the same tool.[11] This intake is approximately 900 to 1,000 calories less than the caloric intake estimated for these active factory workers. Second, the natural variation in adult eating patterns from one period to another may be quite large. Change in occupation or activity may account for some differences. In addition, even though the quality of the diet may not change, the quantity people eat may vary. The dietary interview may pick up these changes and reflect current eating practices and not long range eating patterns.

It is extremely difficult to determine a person's dietary pattern; the only accurate method is actual food analysis. The next most reliable method is to weigh all food consumed. These are obviously not practical measures for epidemiologic studies. Although the dietary interview has shortcomings, it was the most practical method for this study.

SUMMARY

A repeat study, after a two-year interval, was carried out on 147 Italian-American factory workers.

For the group there were no significant changes in serum cholesterol levels or relative weight although some persons showed considerable change.

The mean serum cholesterol level of smokers of one or more packages of cigarettes a day was significantly higher and their relative weight was significantly lower than that of nonsmokers.

The repeat diet histories showed no significant mean differences in calories or grams of fat and protein for the group, but individual variations were found.

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Relationship Between Plasma Amino Acids and Composition of the Ingested Protein

II. A Shortened Procedure to Determine Plasma Amino Acid (PAA) Ratios

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IT has been the desire of nutritionists for many years to develop a direct and short procedure to determine amino acid deficiencies and, in turn, the nutritional quality of a protein. An interrelationship between the concentration changes of the plasma-free amino acids and the amino acid composition of the protein ingested has been demonstrated by an *in vivo* plasma amino acid (PAA) ratio procedure.[1] This interrelationship was suggested by earlier work from this laboratory[2] and by previous reports in the literature.[3–9]

The PAA ratios are determined by expressing the relative changes in concentrations for each of the free essential amino acids of the plasma after the ingestion of a protein meal as a function of the animals' amino acid requirements. This *in vivo* chemical score is feasible by postulating that the free essential amino acids are removed from the plasma by the body tissues at rates proportional to the amino acid requirements of the test animal. Therefore, it is logical to assume that the most limiting amino acid of a dietary protein would give the smallest positive or largest negative ratio, and that the reverse would be true for the least limiting amino acid.

For our feeding studies the general procedure followed has been to take a blood sample immediately before the test meal and at hourly intervals for five hours after its ingestion. The PAA ratio for each amino acid is calculated by subtracting the fasting level amino acid concentration from the average plasma amino acid concentration after the meal. This difference or postabsorptive change is then divided by the amino acid requirement for the test subject and multiplied by 100 to make the ratio a small whole number.

In these initial studies[1] the nutritional limiting orders obtained from the PAA ratios for the essential amino acids of wheat gluten, gelatin and casein for the dog were found to agree quite well with the limiting orders obtained by the chemical score method of Block and Mitchell.[17] By this *in vivo* procedure, lysine was found to be the most limiting amino acid for wheat gluten, tryptophan for gelatin and arginine and methionine for casein.

These experiments demonstrate that amino acid deficiencies of a protein, and proper supplementation levels to overcome these deficiencies can be evaluated by this technic. Opportunities for the quantitative evaluation of amino acids in nutrition by this procedure appear quite promising. It is believed that, with further improvements and advances, this *in vivo* technic will aid in solving a few of the many problems still facing nutritionists.

To increase the utility of this PAA ratio method,[1] modifications which reduce the time and blood volume required for each experiment have been studied and are presented here. The applicability of this procedure for human studies is demonstrated. Preliminary data are also presented which show the general

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TABLE I

Comparison of Plasma Amino Acid Ratios Determined by Long and Short Procedure*

	Methionine	Cystine†	Arginine	Lysine	Phenylalanine‡	Isoleucine	Leucine	Valine	Threonine	Histidine
Long Procedure, Experiment 1 (Part 1)										
Hr. 1	1.04	2.29	3.00	3.09	2.94	1.57	2.80	2.77	4.34	1.81
Hr. 2	1.31	2.67	3.45	2.92	3.05	1.60	2.88	2.96	5.08	1.78
Hr. 3	1.39	2.90	3.49	3.31	3.61	2.00	3.58	3.51	5.30	2.99
Hr. 4	1.12	1.98	2.97	2.90	3.40	1.74	3.16	2.97	5.59	2.77
Hr. 5	1.16	1.76	3.13	3.03	2.84	1.52	2.76	3.05	5.00	1.62
Average (1 to 5)	1.20	2.32	3.21	3.05	3.17	1.69	3.04	3.05	5.06	2.19
Fasting level	0.91	1.67	2.56	2.15	1.98	0.79	1.62	1.81	3.41	1.20
Average minus fasting level	0.29	0.65	0.65	0.90	1.19	0.90	1.42	1.24	1.65	0.99
PAA ratio§	5.7	9.2	11.4	12.7	12.9	14.5	16.7	19.4	41.3	49.5
Limiting order	...	1	2	3	4	5	6	7	8	9
Short Procedure, Experiment 1 (Part 2)										
Pooled (1 to 5)‖	1.13	2.38	3.11	2.98	3.38	1.71	3.21	2.93	4.83	2.33
Fasting level	0.91	1.67	2.56	2.15	1.98	0.79	1.62	1.81	3.41	1.20
Average minus fasting level	0.22	0.71	0.55	0.83	1.40	0.92	1.59	1.12	1.42	1.13
PAA ratio¶	4.3	10.0	9.7	11.7	15.2	14.8	18.7	17.5	35.5	56.5
Limiting order	...	2	1	3	5	4	7	6	8	9

* Dog 96 (23 kg.) fed 84.9 gm. wheat gluten, 93.8 gm. sucrose, 1.28 gm. L-lysine monohydrochloride, and 20 gm. corn oil mixed with 150 ml. H_2O. Amino acid concentrations expressed as mg. per 100 ml. plasma.

† Includes methionine.

‡ Includes tyrosine.

§ Line 8 divided by dog amino acid requirement times 100.[1] Cystine + methionine requirement equals 7.1.[15]

‖ Analysis of pooled sample containing equal volume of five hourly samples after meal.

¶ Line 3 divided by dog amino acid requirement times 100.[1] Cystine + methionine requirement equals 7.1[15]

magnitude of the experimental error encountered in determining the PAA ratios when ion exchange chromatography is employed for amino acid analyses.

EXPERIMENTAL

The general procedure previously reported[1] to determine the PAA ratios has been modified slightly. The outline for a feeding test employing the "long procedure" and the "short procedure" is illustrated by experiment 1, part 1 and experiment 1, part 2, respectively. For experiment 1 (Table I), Dog 96 (23 kg.) ate the wheat gluten test meal after an overnight fast (16 to 18 hours). Immediately before the meal, and each hour for five hours after the meal, 20 ml. of blood was drawn with a heparinized syringe via the jugular vein. Results are nearly identical if blood is drawn via the cephalic vein; however, use of the jugular vein is preferred for large blood samples. The blood samples were centrifuged promptly, and 5 ml. of the plasma for each of the six samples in experiment 1 (part 1, long procedure) was prepared for chromatographic analysis by treating with picric acid.[10] For experiment 1 (part 2, short procedure) the pooled plasma sample was obtained by combining 1 ml. of plasma from each hourly sample in a test tube which was kept in an ice bath. After the fifth-hour sample, the pooled plasma was brought to room temperature and prepared for chromatographic analysis. Keeping the plasma cold in this manner permitted the quantitative recovery of all the amino acids including cystine.

TABLE II
Reference Amino Acid Standard for Adult Human Being

Amino Acid	Ovalbumin* / Threonine = 3.0	Rose† / Threonine = 3.0	Average Reference Standard
Lysine	5.8	4.8	5.3
Methionine	3.3	...	3.3
Cystine‡	4.9	6.6	5.8
Tryptophan	1.1	1.5	1.3
Phenylalanine§	7.7	6.6	7.2
Threonine	3.0	3.0	3.0
Isoleucine	4.2	4.2	4.2
Leucine	6.4	6.6	6.5
Valine	4.8	4.8	4.8

* Triplicate chromatographic analysis. Ovalbumin obtained from Joe Lowe Corp., New York.
† Rose minimum requirements.[13]
‡ Includes methionine.
§ Includes tyrosine.

A recently developed chromatographic method of Hamilton[11] is now being employed for the amino acid analyses. This method is faster and eliminates repacking of the columns, which was necessary in the procedure previously employed.[12] An additional saving of time has been realized by modifying the photometric ninhydrin procedure of Moore and Stein.[16] After the ninhydrin color is developed and each fraction is diluted with the alcohol-water mixture, the tubes for each peak are poured into an amber Erlenmeyer flask and mixed thoroughly. A Beckman Model DU Spectrophotometer is employed to determine the optical density for each individual amino acid peak. With the use of standards it has been found that this procedure is as accurate as reading each individual fraction.

In utilizing the "long" or "short" procedures a minimum of 30 ml. and 10 ml., respectively, of plasma is required for a feeding test. If one also desired to determine tryptophan, an additional 40 per cent plasma volume is required in each case since microbiological assay is employed for this analysis.

For the human feeding tests, the method outlined for the dog studies was followed as closely as possible. All human subjects were normal, healthy, adult males. No restriction was placed on the subject's diet before the test, except that he consumed nothing but water sixteen hours prior to the experiment. For easy consumption of the test meal, the test protein (wheat gluten) and corn oil were slurried in small portions of orange juice and consumed as rapidly after mixing as possible. For each experiment a total of 150 gm. of a frozen orange juice concentrate diluted to 500 ml. was consumed. Usually, the meal was ingested in two to three minutes. Blood was obtained at the specified times by venous puncture in the cubital region, and the plasma obtained and analyzed as described for the dog. The long procedure was employed for experiments 2, 3 and 4 (Table III) and the short procedure was used for experiments 5 and 6 (Table IV). Replicate experiments with the same subject were conducted with a three- to six-week period between tests to be certain that no stress due to blood volume loss could be placed upon the subject. This precaution has been followed since it has been observed during the course of our studies (unpublished data), that a stress apparently due to blood volume loss may cause a general lowering of the PAA ratios. For all the experiments reported here, the PAA ratios for methionine alone and for methionine plus cystine are given, with the latter being used in estimating the nutritional limiting order. Both are presented since there is some question as to which method of calculation should be preferred.

RESULTS AND COMMENTS

The results of a feeding test with wheat gluten supplemented with L-lysine monohydrochloride for Dog 96 are given in Table I. In part 1 of this experiment, the long procedure employed previously[1] with certain modifications (see Experimental section) was used to determine the PAA ratios and the *in vivo* nutritional limiting order. The same values were determined in part 2 by the short procedure (see Experimental section). It can be seen that variations between the PAA ratios obtained by the two procedures are within the experimental error of the method employed for the amino acid analyses.

These findings demonstrate that the short

TABLE III

Triplicate Human Studies with Wheat Gluten* Giving Standard Deviations for PAA Ratios

	Lysine	Methionine	Cystine†	Tryptophan	Phenylalanine‡	Threonine	Isoleucine	Leucine	Valine
Experiment 2 (Subject A)									
Hr. 1	2.48	0.75	1.88	1.32	3.23	2.30	2.12	3.57	4.29
Hr. 2	2.86	0.85	2.06	1.32	3.85	2.90	2.72	4.59	5.69
Hr. 3	2.32	0.74	1.92	1.30	3.81	2.21	2.38	4.02	5.59
Hr. 4	2.58	0.71	2.03	1.24	2.68	2.35	2.41	4.10	5.56
Hr. 5	2.00	0.51	1.70	1.24	3.03	1.95	1.77	3.01	4.89
Average (1 to 5)	2.45	0.71	1.92	1.28	3.32	2.34	2.28	3.85	5.20
Fasting level	2.46	0.57	1.40	1.20	2.25	1.52	1.15	2.00	3.00
Average minus fasting level	−0.01	0.14	0.52	0.08	1.07	0.82	1.13	1.85	2.20
PAA ratio§	−0.2	4.2	9.0	6.2	14.9	27.5	26.9	28.5	45.9
Limiting order	1	...	3	2	4	6	5	7	8
Experiment 3 (Subject A)									
Hr. 1	2.39	0.58	2.38	1.56	2.89	2.28	2.15	3.44	4.70
Hr. 2	2.43	0.63	1.59	1.76	3.29	2.33	2.30	3.86	4.87
Hr. 3	2.34	0.76	2.12	1.75	4.16	2.45	2.38	4.08	5.68
Hr. 4	1.87	0.74	1.99	1.48	2.77	2.55	2.11	3.52	4.76
Hr. 5	1.93	0.52	1.46	1.18	2.55	1.69	1.60	2.99	3.75
Average (1 to 5)	2.19	0.65	1.91	1.55	3.13	2.26	2.11	3.58	4.75
Fasting level	2.34	0.40	1.55	1.37	1.71	1.65	1.00	1.88	3.07
Average minus fasting level	−0.15	0.25	0.45	0.18	1.42	0.61	1.11	1.70	1.68
PAA ratio§	−2.8	7.6	7.8	13.9	19.7	20.3	26.4	26.2	35.0
Limiting order	1	...	2	3	4	5	7	6	8
Experiment 4 (Subject A)									
Hr. 1	2.52	0.73	2.48	1.49	3.84	2.22	2.06	3.24	3.96
Hr. 2	3.28	0.60	1.10	1.85	2.78	2.88	2.07	3.40	4.04
Hr. 3	2.38	0.73	2.28	1.79	4.70	2.27	2.24	3.69	4.52
Hr. 4	2.29	0.51	1.80	1.33	3.00	2.14	1.73	3.02	6.10
Hr. 5	1.94	0.49	1.89	1.37	3.50	2.33	1.44	3.28	4.18
Average (1 to 5)	2.48	0.61	1.91	1.57	3.56	2.37	1.91	3.33	4.70
Fasting level	2.56	0.43	1.45	1.38	2.16	1.76	1.09	1.93	3.15
Average minus fasting level	−0.08	0.18	0.46	0.19	1.40	0.61	0.82	1.37	1.55
PAA ratio§	−1.5	5.5	7.9	16.1	19.4	20.3	19.5	21.1	32.3
Limiting order	1	...	2	3	4	6	5	7	8
Average PAA ratio	−1.5	5.8	8.2	12.1	18.0	22.7	24.3	25.3	37.7
± standard deviation	±1.3	±1.7	±0.7	±5.2	±2.7	±4.4	±2.6	±3.8	±7.2
Limiting order	1	...	2	3	4	5	6	7	8
Chemical score wheat gluten‖	35.9	48.5	59.5	69.2	131.0	96.7	105.0	117.0	91.5
Limiting order	1	...	2	3	8	5	6	7	4

* Subject A (80 kg.) fed 84.9 gm. wheat gluten and 20 gm. oil slurred in 500 ml. of orange juice. Amino acid concentrations expressed as mg. per 100 ml.

† Includes methionine.

‡ Includes tyrosine.

§ Line 8 divided by amino acid reference standard (Table II) times 100.

‖ Scored against amino acid reference standard (Table II). Amino acid and protein content of wheat gluten previously given.[1]

TABLE IV
Variation of PAA Ratios for Human Subjects A* and B*

Experiment No.	Lysine	Methionine	Cystine†	Tryptophan	Phenylalanine‡	Threonine	Isoleucine	Leucine	Valine
				PAA Ratio§					
5‖ (Subject B).	−1.1	9.4	13.8	40.2	33.8	40.0	31.9	35.2	47.6
6‖ (Subject B).	2.3	13.6	15.0	29.4	35.0	45.7	33.8	35.1	49.8
5 and 6.....	0.6	11.5	14.4	34.8	34.4	42.9	32.9	35.2	48.7
Average ± standard deviation...	±2.2	±3.0	±0.9	±7.6	±0.9	±4.0	±1.3	±0.2	±1.6
2, 3 and 4¶ (Subject A).	−1.5	5.8	8.2	12.1	18.0	22.7	24.3	25.3	37.7
Average ± standard deviation...	±1.3	±1.7	±0.7	±5.2	±2.7	±4.4	±2.6	±3.8	±7.2
Weighted standard deviation 2 through 6...	±1.5	±2.3	±0.8	±6.3	±2.2	±4.1	±2.2	±2.9	±5.7

* Both subjects weighed 80 kg. and consumed 84.9 gm. wheat gluten and 20 gm. corn oil slurried in 500 ml. of orange juice.
† Includes methionine.
‡ Includes tyrosine.
§ Calculated as shown in Table III.
‖ Short procedure employed.
¶ Data from Table III.

procedure can be utilized to determine PAA ratios with a 60 to 70 per cent saving in time and blood volume required for each test. The use of this short method will also reduce the possibility of stress due to blood volume loss being placed upon the subject. Development of this method should permit its use on small animals. Preliminary tests on individual animals or groups of animals would be necessary to establish the proper timing for taking the blood samples after the meal. Having established this, the short procedure could be employed to conduct the complete test.

Triplicate human feeding studies with wheat gluten (experiments 2, 3 and 4) were conducted to determine whether (1) the PAA ratio procedure could be utilized for human studies and (2) the PAA ratios for the same subject and protein were reproducible. For the human experiments an amino acid "reference standard" was obtained by averaging the amino acid pattern for ovalbumin and the minimum amino acid requirements determined by Rose[13] with threonine set at 3.0 (Table II). Using this reference standard for the human studies, the PAA ratios were calculated as reported for the dog.[1]

A tabulation of all the data for these three experiments is shown in Table III. A striking similarity between the plasma amino acid changes after the meal and the PAA ratios for the three experiments is shown. Two-way analyses of variance[14] and calculated 95 per cent confidence limits of the experimental data presented here revealed that the general nutritional limiting order determined from the PAA ratios is statistically sound. Of greatest interest are the most limiting amino acids, and analyses reveal that statistical significance can be given to the PAA ratios for these amino acids. These findings parallel those found for the dog[1] and show the general reproducibility of the PAA ratios and the usefulness of this method for human studies.

Two striking differences are noted between the nutritional limiting order obtained from

the PAA ratios for experiments 2, 3 and 4 and the chemical score of wheat gluten. From the nutritional limiting order, phenylalanine appears to be more limiting and valine less limiting than indicated by the chemical score. These findings probably reflect differences in availability and rate of absorption.

To compare the variation of the PAA ratios for different subjects with that obtained for the same subject, experiments 5 and 6 were conducted with subject B. Both human subjects (A and B) weighed 80 kg. and were fed the identical wheat gluten test meals. The average PAA ratios with the standard deviations are given in Table IV for the triplicate tests with subject A (experiment 2, 3 and 4) and for the duplicate tests with subject B (experiment 5 and 6). Also given is the weighted standard deviation for each amino acid obtained by pooling the results for the five individual experiments. The variability in the PAA ratios for each amino acid for different subjects is similar even though the ratios may be different.

Comparison of the average PAA ratios for the two subjects shows the ratios to be generally higher for subject B than subject A. In particular statistical significance at the 95 per cent level can be given to the differences in the ratios for the two subjects for tryptophan, phenylalanine plus tyrosine, threonine, isoleucine and leucine. It appears also that significance should be given to the differences in the ratios for the two subjects for cystine plus methionine. However, since the standard deviation found for this amino acid pair is unusually low compared to that found for the other amino acids, reservation will be exercised in claiming significance in this case until additional studies can confirm or reject this standard deviation value. Variations of this nature in the PAA ratios that appear for normal subjects may reflect differences in protein utilization and/or amino acid requirements and/or protein nutritional status. Utilization of this technic with subjects under stress, e.g., illness, malnutrition, may serve to uncover relationships between protein and amino acid metabolism under certain particular stress conditions.

SUMMARY

A previous report[1] presented an *in vivo* plasma amino acid (PAA) ratio technic to evaluate the amino acid adequacy in a food protein for the nourishment of the dog. A modification of the original method is presented which reduces by approximately 60 per cent the time and amount of blood required for each experiment. The applicability of this procedure for human studies is demonstrated. The reproducibility of the PAA ratios is shown with the variation from subject to subject being significantly greater for some amino acids than the variation for the same subject. These findings may reflect individual differences in protein utilization and/or amino acid requirements and/or protein nutritional status. Experimentation with subjects under stress, e.g., illness, malnutrition, should be quite promising.

ACKNOWLEDGMENT

We wish to thank Louise B. Roemer for technical assistance in these studies. We also wish to thank the du Pont Haskell Laboratory for placing at our disposal the necessary personnel and laboratory facilities to carry out these studies.

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Reviews of Recent Books

Modern Scientific Aspects of Neurology, edited by J. N. Cumings. Edward Arnold Ltd. London, 1960. (Williams & Wilkins, American Agents), pp. 360 $13.00.

Neurology long ago left the bedside for the laboratory. In this book ten authors discuss "scientific aspects" of neurology, i.e., biochemistry, enzymology, electron microscopy, etc. Both lipids and nucleic acids come in for special attention and it is gratifying to see how much is known—at least in the laboratory—about the brain and its appendages. Unfortunately many neurologic disorders, especially the demyelinating diseases, continue to baffle the scientist and injure the patient.

As a review of current knowledge of the basic sciences as related to neurologic medicine, this book can be recommended highly. It should be especially useful to workers in the field. J. H. S.

Biochemical Values in Clinical Medicine. The Results Following Pathological or Psychological Change, by Robert D. Eastham. John Wright & Sons, Bristol, 1960 (Williams & Wilkins Co., American agents), pp. 144, $3.75.

This little pocket-sized manual summarizes all the important laboratory procedures by giving the normal values and the clinical conditions which produce changes in them. If you want to know what disorders are associated with an increase in lactic acid dehydrogenase in the serum, or something about the "pyruvate metabolism test," you will find it in this book. A useful feature is the bibliographic reference with each test. This book should prove to be popular. S. O. W.

Aids to Biochemistry, by S. P. Datta and J. H. Ottaway. Bailliere, Tindall and Cox, London (Williams & Wilkins Co., Baltimore, American Agents), 1960, pp. 266, $3.75.

In a recent article[1] in the British *Lancet* an American teacher of biochemistry summarized his impressions of the position of biochemistry in the medical curriculum in London medical schools. He points out that anatomy is by far the major subject of the preclinical curriculum while biochemistry is a minor one. In response to the immediate and overt pressures of the former subject, the student is reluctant to commit valuable and limited time and brain-space to biochemistry. "A talented note-taker he does not listen to lectures or read biochemistry"; his lecture notes may be revised and studied just before trial examinations while his "capacity for cramming is impressive." The London faculty, according to Dr. Schein, thought that the encyclopedic American textbooks of biochemistry were too detailed, high powered and expensive and would not be read by the students.

These and other interesting comments were recalled as I examined the pocket-sized "Aids to Biochemistry," now in its fifth edition. Its brevity, scope, didactic approach and indeed its title indicate that its primary purpose is to supplement lectures and to afford a quick review of the subject. Covering a tremendous range in its small size, the material, on the whole, is up to date and carefully sifted to present basic information in preclinical biochemistry. As such there is no clinical orientation, this, presumably being left to another "aid" in "abnormal" biochemistry (chemical pathology) taught in the clinical years of school.

American medical students, with their myriad of pressures and unevenness in chemistry backgrounds and interests in basic science, are often not averse to using synoptic types of biochemistry books. Perhaps in similar proportion to the weight and volume of our recommended texts, our American synopses are larger than their English counterparts. Unfortunately, they too are unable to help give the student the stimulus toward greater interest and understanding of the key role of biochemistry in modern medicine so that he will want to expand his knowledge *after* he passes the necessary qualifying examinations.

MAURICE E. SHILS

The Chemistry of Lipids in Health and Disease, by H. K. King, PH.D., Charles C Thomas, Springfield, Ill., 1960, pp. 102, $3.75.

The author is a Senior Lecturer in Biochemistry at The University of Liverpool. The title of the monograph is somewhat misleading for it only gives a brief account of some biochemical aspects of lipids in health and in atherosclerosis. Apparently the monograph is based on his lectures and may therefore serve well for orientation of medical students.

The author chooses to treat the subject in broad outline and without detailed documentation. The monograph would be of little help to those who are looking for a detailed guide to the literature. However, it does accomplish what the author intended—it gives the reader a background against which he may read and judge the current literature.

G. C. CHIU

[1] SCHEIN, A. H. Preclinical biochemistry. A view from London Bridge. *Lancet*, 1: 691 1960.

Cirrhosis of the Liver, by Martin Seler Kleckner, Jr. Charles C Thomas, Publisher, Springfield, Ill., 1960, pp. 729, $24.50.

This book is an exhaustive survey of the literature bearing on a disease of vital importance to the clinician. In this grandly illustrated text, the author has succeeded in bringing together a wealth of material on experimental and clinical cirrhosis. The section on morphology, including as it does the well known stereograms of Hans Elias, is particularly valuable. Chapters dealing with the various types of cirrhosis and the three sections on portal hypertension, ascites and hepatic insufficiency are competently handled.

Two annoying defects detract from the value of this work. The author frequently documents long series by "tossing in" references as a body at the end of the paragraph. A listing of some twenty-two causes of hepatic coma, for example, is followed at the end of the paragraph by thirty-three individual references bearing no relation to the order of the series. The reader is then left the chore of mating statement and source for statement. Secondly, the three-page index is shockingly inadequate and really nothing more than an alphabetized table of contents. It is regrettable that such faults should mar this otherwise admirable treatise.

J. E. WING

BOOKS RECEIVED FOR REVIEW

Books received for review by THE AMERICAN JOURNAL OF CLINICAL NUTRITION are acknowledged in this column. As far as practicable, those of special interest are selected, as space permits, for extensive review.

Transcript of the Workshop on Bone Densitometry, by Stanley M. Garn, Harold E. Harrison and Karl E. Mason. Fels Research Institute for the Study of Human Development, Yellow Springs, Ohio, 1960, pp. 240.

Dietary Proteins in Health and Disease, by James B. Allison and William H. Fitzpatrick. Charles C Thomas, Springfield, 1960, pp. 86, $4.50.

Chemicals in Food and in Farm Product: Their Harmful Effects, by Franklin Bicknell. Faber & Faber, London, 1960, pp. 192, 12*s* 6*d*.

Clinical Studies in Nutrition, by Eleanora Sense. J. B. Lippincott Company, Philadelphia, 1960, pp. 249, $4.00.

Ciba Foundation Study Group No. 5. Regulation of the Inorganic Ion Content of Cells, edited by G. E. W. Wolstenholme and C. M. O'Connor. Little, Brown & Company, Boston, 1960, pp. 100.

Inter-African Nutrition Conference, Third Session, Vol. II. Inter-African Conference of Nutrition, Angola, Portugal, 1956, pp. 889.

Hypothermia and the Effects of Cold. British Medical Bulletin, Vol. 17, No. 1. The British Council, London, January, 1961, $3.25.

The National Library of Medicine Index Mechanization Project. Bulletin of the Medical Library Association, Vol. 49, No. 1. National Library of Medicine, Washington, D. C., 1961, pp. 96.

Food Additive Control in the United Kingdom, by C. L. Hinton. FAO, Italy, 1960 (Columbia University Press, American Agents), pp. 52.

Nutrition News

The Gordon Research Conference on Food and Nutrition

The Gordon Research Conference on Food and Nutrition will be held at Colby Junior College, New London, New Hampshire, on August 21–25, 1961. The topics for discussion include Food Additives, Special Problems, Fatty Acids and Tocopherol, Carbohydrate and Nutrient Utilization, Nutrition and Disease, Future Food Problems, Obesity and Atherosclerosis. Chairmen of these sections are, respectively: Bernard L. Oser, Herbert Pollack, Robert Harris, Z. I. Kertesz, N. S. Scrimshaw, Paul B. Pearson and Theodore Van Itallie. For further information write to W. George Parker, M.D., Department of Chemistry, University of Rhode Island, Kingston, R. I.

Abstracts of Current Literature

CHARLES R. SHUMAN, M.D., EDITOR

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ADRENAL STEROIDS AND NUTRITION

The biochemical effects of adrenal steroids influence the nutrition status in many ways. Protein, fat, carbohydrate and electrolyte metabolism are altered by administration of these hormones. One of the biochemical lesions which has received considerable attention is that of hypokalemic alkalosis.

Metabolic Alkalosis. A Specific Effect of Adrenocortical Hormones. A. P. Grollman and J. L. Gamble, Jr. *Am. J. Physiol.*, 196: 135, 1959.

With appropriate balance experiments and skeletal muscle analyses, it was shown that (1) in dogs placed on a potassium-restricted diet approximately 15 per cent of their muscle cell potassium was lost but alkalosis did not develop; (2) the administration of DCA, ACTH or cortisone to animals on a potassium-restricted diet evoked metabolic alkalosis which was sustained only as long as the hormones were administered; a prompt return to normal occurred despite continued potassium restriction; (3) alkalosis induced by chloride withdrawal or by cortisone administration during complete sodium restriction was not associated with evidence of potassium loss. These findings indicate that the increase in the serum bicarbonate induced by the steroids is a relatively direct effect, one that cannot be dependable related in a reciprocal fashion to the associated losses of body potassium. AUTHORS

Hirsutism in females has been attributed to alterations in the formation of adrenal steroids resulting in an increase in certain 17-ketosteroid fractions. Hypertension in hirsute females has been ascribed to a defect in 11 beta-hydroxylase activity with increased concentrations of compound S. The effect of prednisone in suppressing ACTH release may have a salutary influence.

The Metabolism of Steroids of the Adrenal Cortex in Idiopathic Hirsutism. F. De Ritis, M. Minozzi, M. Faggiano and D. Napolitani. *Ztschr. Vitamin Hormon- u. Fermentforsch.*, 10: 1, 1959.

An increase in the urinary excretion of 17-ketosteroids, involving all fractions, was evident in two of twelve women with hirsutism. These cases are ascribed to a rise in the production of androgens in the adrenal cortex. In seven women, of whom only three showed excessive 17-ketosteroid excretion, the fourth and fifth fractions were increased. Here there is probably a relative insufficiency of 11β-hydroxylase, as a result of which certain glucocorticoid precursors accumulate and are then converted into 17-ketosteroids exerting an androgenic action. This is presumably the mechanism responsible for ordinary hirsutism, i.e., hirsutism unaccompanied by any other signs of endocrine disorder. In one case of hirsutism in which the hormonal balance showed no deviation whatever from normal, the condition is attributed to the fact that certain tissues react abnormally to androgens. AUTHORS

The hypothalamic control of aldosterone release from adrenal cortex has been postulated. Subcortical nuclear centers sensitive to changes in arterial blood volume or in blood electrolyte concentration have been proposed as the principal factors responsible for the control of this hormone secretion through neurohumoral substances.

Cerebral Regulation of Sodium Excretion. A. G. B. Kovách, M. Földi, N. Papp, P. S. Róheim and E. Koltay. *Lancet*, 1: 338, 1959.

In the experiments briefly reported here from Budapest, a particularly difficult animal preparation was necessary. The head of a dog is vascularly isolated from its body and maintained by crossed circulation

from another dog. Its only connection with its own body is by nerves and the spinal cord.

Injection of a mannitol solution into the carotid artery of the head caused no significant increase in the sodium excretion of its isolated body. The injection of 5.4 per cent sodium phosphate buffer solution increased sodium excretion to 196 per cent of control values, without altering water excretion, sodium filtration rate or potassium excretion. If the kidneys were denervated, injection of hypertonic sodium solution increased sodium excretion to about 250 per cent of control values with increased water secretion. If the adrenals were removed, no increase in sodium occurred. It is concluded that sodium excretion is controlled by central nervous impulses acting through the adrenals. Whether antidiuretic hormone is the primary influence which first affects the central nervous system is not yet known.

F. E. HYTTEN

A Direct Influence of 5-Hydroxytryptamine on the Adrenal Cortex. H. Rosenkrantz. *Endocrinology*, 64: 355, 1959.

In the experiments described in this paper, it has been demonstrated that serotonin has a direct effect on the adrenal cortex. Following incubation of serotonin with mammalian adrenal tissue, there was an increased release of material which reacted with blue tetrazolium. The substances released by the adrenal in response to serotonin appear to be steroidal in nature since on paper and column chromatography they moved at about the rate of cortisol and cortisone. Furthermore, this material exhibited sodium-retaining activity which was greater than that of desoxycorticosterone. Although it is likely that the compounds released by the adrenal after exposure to serotonin are steroids, it has not been proved that this is so. Further studies of the influence of serotonin on the adrenal glands are indicated.

A. B. EISENSTEIN

Many derivatives of cortisone have been produced and subjected to extensive investigation in an effort to develop a substance with maximum therapeutic efficacy and minimum undesirable side effects.

Adrenal Cortical Activities of 9a-fluoro-11β,16a, 17a, 21-tetrahydroxy-1,4-Pregnadiene-3,20-Dione. J. W. Perrine, L. Bortle, E. Heyder, R. Partridge, E. K. Ross and I. Ringler. *Endocrinology*, 64: 437, 1959.

The biologic activity of a new synthetic steroid (Triamcinolone®) has been compared with that of hydrocortisone. Triamcinolone is a compound in which several alterations in molecular structure have been made. These structural changes include introduction of unsaturated bonds between C-1 and C-2, attachment of a fluorine substituent at the 9-alpha position, and addition of a 16-alpha hydroxyl group. Triamcinolone has been shown to be far more potent than hydrocortisone in causing liver glycogen deposition and also demonstrates a marked increase in anti-inflammatory activity. It is of particular interest that administration of this compound results in sodium and water diuresis. The synthesis of highly potent adrenal steroid compounds which do not cause hypertension and edema represents a significant advance in the therapy of a variety of diseases.

A. B. EISENSTEIN

In Vitro and In Vivo Metabolism of Prednisolone: Studies Concerning Its Biological Effectiveness. E. M. Glenn. *Endocrinology*, 64: 373, 1959.

This study was carried out to determine whether degradation of prednisolone is less rapid than that of naturally occurring corticosteroids and, if so, whether delayed inactivation can account for increased metabolic activity of the synthetic compound. In vitro studies showed that reduction of both the alpha-ketol side chain and the alpha-beta, unsaturated ketone structure of prednisolone were considerably slower than reduction of these substituents in the hydrocortisone molecule. Increased liver glycogen deposition following prednisolone administration was of greater duration than that which followed hydrocortisone. Following intraperitoneal injection of prednisolone, relatively large quantities of the unaltered compound were excreted in the urine. In contrast, after intraperitoneal injection of an equivalent dose of hydrocortisone, the urine contained only small amounts of this hormone. Although these experiments clearly demonstrated that prednisolone is degraded much less readily than hydrocortisone, it has not been proved that this accounts for the greater potency of the synthetic steroid. Despite the lack of conclusive proof, the hypothesis that metabolic activity of a hormone may depend in part on its rate of degradation remains attractive.

A. B. EISENSTEIN

LIVER DISEASE: NUTRITIONAL AND ALCOHOLIC

The development of portal cirrhosis is usually an evanescent and slowly progressive condition. The morphologic classification of this disease described subsequently is an important contribution which may be correlated with the clinical findings in specific categories and which may have precise therapeutic and prognostic implications.

Precision in the Classification of Cirrhosis of the Liver. P. E. Steiner, *Am. J. Path.*, 37: 21, 1960.

Based on comparative studies of livers of patients in various geographic and ethnic groups "cirrhosis" is classified on morphologic grounds into postnecrotic portal and florid types. Postnecrotic cirrhosis is characterized by evidence of preceding lobular collapse, and is indicated by irregular scarring and crowding of three or more portal triads within individual areas of connective tissue. Portal cirrhosis usually shows no scarring due to lobular collapse and is subdivided into four subgroups: (1) simple cirrhosis, (2) simple cirrhosis with areas of questionable collapse, (3) cirrhosis with

fatty change, and (4) cirrhosis with fatty change and fibrosis which may extend into individual nodules. Florid cirrhosis may be recognized by nodular hyperplasia, large fat droplets and the presence of inflammatory cells within lobules and septums. These changes are often associated with liver cell necrosis. The fibrosis is intra- and monolobular. This is a genuine cirrhosis because of new formation of fibrous tissue, nodular regeneration and distortion of the liver pattern. The role of fat in the evolution of cirrhosis in man is left in doubt. However, in contrast with the views held by other investigators it is believed that fat plays a secondary role and that it is desirable to separate fatty from non-fatty cirrhosis as long as the role of fat in the pathogenesis of this disease entity is not clearly established.

M. SILBERBERG

Alcohol may damage the liver directly as a toxic substance or by interfering with nutrition as the principal source of calories to the exclusion of protein and essential food factors. There is evidence favoring both points of view and it seems probable that each of these effects of alcohol ingestion plays a role in hepatic damage.

Observations on the Histogenesis of Cirrhosis of the Liver in Chronic Alcoholism. R. G. Shorter and A. H. Baggenstoss, *Am. J. Clin. Path.*, 32: 422, 1959.

The livers of seventy-one patients with a proved history of chronic alcoholism but without cirrhosis and those of eighty-two alcoholics with cirrhosis were studied. Most of these patients had been whiskey drinkers from one to fifty years. Of the seventy-one without cirrhosis sixty-seven were men (twenty-four to eighty-six years of age) and four were women (forty-one to seventy-one years old). Of the eighty-two cirrhotic subjects seventy-two were men (twenty-eight to seventy-four years of age) and ten were women (thirty-four to seventy-five years old). In the livers of sixty-five patients without cirrhosis there were foci of lysis and necrosis; such foci were found in all cases of cirrhosis. Mallory's "alcoholic hyalin" was observed in the cytoplasm of twenty-three patients without cirrhosis and of sixty-five with cirrhosis. Fatty cysts were found in a total of seven cases; they were associated with condensation of reticulin, but not with formation of collagen. Both stromal collapse and condensation of reticulin were more conspicuous in relation to necrotic foci than to areas of fatty change. It is thought that hepatic necrosis with stromal collapse is conducive to nodular regeneration of liver tissue. This process is associated with changes in the vascular pattern leading ultimately to cirrhosis.

M. SILBERBERG

Cirrhosis of the Liver: A Study of Alcoholic and Nonalcoholic Patients in Boston and London. W. H. J. Summerskill, C. S. Davidson, J. H. Dible, G. K. Mallory, S. Sherlock, M. D. Turner and S. J. Wolfe. *New England J. Med.*, 262: 1, 1960.

In this study thirty-five alcoholic patients with cirrhosis of the liver studied in Boston are compared with thirty-five patients with cirrhosis due to unknown causes studied in a London hospital. Clinical and pathologic findings are presented and compared in the two groups. Among the many observations made were that hepatitis is often found in the non-alcoholic group while a history of hepatitis is not often found in the alcoholic patients. A deterioration of liver function is the most common factor in the alcoholic group while non-alcoholic patients more frequently show clinical features related to portal hypertension.

It is not clear why two groups of different social, economic and ethnic composition from two different countries were used in the study. The authors, however, did not believe that the clinical and pathological differences found between the two groups could be explained by differences in locale.

M. W. BATES

"Alcoholic" Hyalin in Human Cirrhosis: Histochemical Studies. S. A. Nozkin, R. Weitzel, D. Campagna-Pinto, A. MacDonald and G. K. Mallory, *Am. J. Path.*, 37: 49, 1960.

Fatty cirrhotic livers of seventy-four alcoholic patients were studied microscopically and histochemically by means of light and phase microscopy. The material was secured from necropsies or liver punch biopsies. The chemical composition of the "alcoholic" hyalin was analyzed by a variety of routine stains, and special stains were made to demonstrate the possible presence of such pigments as bile, blood, ceroid, etc. In fixed and frozen-dried tissue, proteins, carbohydrates and lipids were studied. In addition, enzymatic investigations were carried out in regard to alkaline and acid phosphatase, lipase, esterase, 5-nucleotidase and succinic dehydrogenase. Enzymatic activities could not be demonstrated in the hyalin. An evaluation of the various staining procedures led to the conclusion that the intracytoplasmic "alcoholic" hyalin seen in cirrhotic livers seems to be an insoluble basic protein complex or a denatured protein formed in living liver cells.

M. SILBERBERG

Liver Dehydrogenase Activity in Chronic Alcoholism. A Comparative Histochemical Study. S. W. French, *Arch. Path.*, 69: 303, 1960.

Livers were studied histologically and histochemically in twenty-five alcoholic patients thirty-three to sixty-six years old (twelve male and thirteen female); two were Negroes and twenty-three were white. Livers of twenty-four non-alcoholic subjects fourteen to eighty years old (fifteen male and nine female), four of which were Negroes, two orientals and eighteen whites, served as controls. The material was obtained by laparotomy, needle biopsies or at necropsy. Besides histologic studies, tissue slices were tested as to their content of diphosphopyridine nucleotide dehydrogenase (DPNHD) and of succinic dehydrogenase (SHD). In livers that had undergone fatty change only, DPNHD was focally absent from the liver cords while SHD was un-

changed. In livers in which interlobular fibrosis, alcoholic hyalin degeneration and fat-laden regenerating liver nodules indicated advanced cirrhotic change, both enzymes were absent. These findings are thought to be due partly to the state of malnutrition of the patient or to focal ischemia, and probably constitute a sign of relative or absolute deficiency of riboflavin.

M. SILBERBERG

Effect of Ethanol Administration on Brain and Liver Enzyme Activities. F. W. Kinard and M. G. Hay. *Am. J. Physiol.*, 198: 657, 1960.

Rats receiving daily administrations of ethanol for ten to twelve weeks became demonstrably resistant to intoxicating effects of the alcohol by the ninth week. The increased resistance to the effects of alcohol was not accompanied by increased oxidation of alcohol in the intact animal or by increased oxidation of ethanol in liver homogenate. Brain AChE activity is reduced to a statistically demonstrable but functionally insignificant degree by administration of alcohol whether the rat is receiving its first dose or whether it has become tolerant to the intoxicating effects of alcohol. The development of tolerance to ethanol appears to be unrelated to the rate of oxidation of alcohol in either the intact animal or in liver tissue and does not appear related to changes in brain AChE activity. AUTHORS

The effect of liver disease on vitamin B_{12} levels in serum and hepatic tissue is illustrated in the following investigations.

Hepatic and Serum Vitamin B_{12} Content in Liver Disease. R. S. Nelson and V. M. Doctor. *Gastroenterology*, 38: 188, 1960.

The vitamin B_{12} content of serum and fresh liver biopsy specimens was determined by bioassay. The average concentration of vitamin B_{12} in liver was lower than the control group in patients with cirrhosis, fatty metamorphosis, intrahepatic cholestasis, obstructive jaundice and viral hepatitis. Lowest values were seen in those with fatty metamorphosis, fatty cirrhosis and viral hepatitis in the convalescent stage.

Serum vitamin B_{12} levels were within normal limits in all patients with fatty metamorphosis, but there were occasional elevations in all other groups. Serum concentrations were increased in all patients with viral hepatitis in the acute phase.

There was no definite correlation between elevated serum vitamin B_{12} values and inflammatory changes in the liver, since diseases not associated with inflammation were occasionally the cause of serum elevations. Impaired ability of the liver to store vitamin B_{12} as well as release of the vitamin during cellular necrosis appeared to be the most important factors influencing serum and liver concentrations in hepatic disease.

J. B. HAMMOND

Serum Vitamin B_{12} Levels in Liver Disease. D. C. Cowling and I. R. Mackay. *M. J. Australia*, 2: 558, 1959.

Microbiologic assay of serum vitamin B_{12} in fifty-seven cases of human liver disease demonstrated raised values in neoplastic and diffuse parenchymatous conditions. The increased levels were chiefly attributable to the release of stored vitamin B_{12} from necrotic hepatic cells and to the increase in vitamin B_{12}-binding capacity of serum proteins. However, the serial serum vitamin B_{12} levels did not correlate on occasion with the results of liver biopsy and plasma glutamic oxalacetic transaminase values. Further, since microbiologic assay is slow and time-consuming, the authors concluded that this indicator has little practical value in the assessment of the nature and severity of liver disease.

R. E. BERNSTEIN

Functional disturbances in the liver alter the metabolism of carbohydrate, fat and protein. In liver disease there is impairment of the utilization of carbohydrate, of the uptake of fatty acid and, as illustrated in the following study, of the disposition of amino acids.

Amino Acids in Arterial and Hepatic Venous Blood of Patients with Laennec's Cirrhosis. S. M. Mellinkoff, T. B. Reynolds, M. Frankland and M. Greipel. *Gastroenterology*, 36: 780, 1959.

The authors determined the amino acid concentration in hepatic vein blood obtained by catheterization and in femoral artery blood in fourteen patients with Laennec's cirrhosis. The hepatic vein blood contained increased concentrations of glutamic acid and decreased concentrations of alpha-alanine. These changes did not occur in every patient, and there were other changes of equal magnitude occurring in only one to several patients. The intravenous injection of mixtures of amino acids and the ingestion of milk greatly altered the relative blood amino acid concentrations. No change in the distribution of amino acids followed the depression of liver blood flow of 20 to 40 per cent by injections of pitressin.

It was concluded that the liver may add to, or remove from, incoming blood various amino acids separately according to metabolic needs. J. B. HAMMOND

A new technic for the study of dynamics of the proliferative fibrous changes observed in portal cirrhosis has been introduced.

Fibrous Tissue in Nutritional Cirrhosis: Autoradiographic Studies with Use of Tritiated Thymidine. R. A. MacDonald and G. K. Mallory. *Arch. Path.*, 67: 119, 1959.

Rats of the Sprague-Dawley strain about two months old were fed a choline-deficient diet ad libitum. After four to eight months, when fatty changes and fibrosis of the liver had developed, the animals were given intraperitoneal injections of 1.0 μc. per gm. body weight of

tritiated thymidine, and sacrificed about four hours later. Thymidine was used in order to determine whether or not it might be taken up by liver and stromal cells of the cirrhotic animals. Since DNA and its precursors are thought to indicate growth and mitotic activity, a positive finding might be interpreted as evidence that in the evolution of nutritional liver cirrhosis one has to deal with an active new formation of connective tissue rather than collapse fibrosis following injury to the liver parenchyma. The livers from these cirrhotic animals were studied by means of various histologic and histochemical methods, and of autoradiography, and compared with conditions found in control animals fed a fat diet for a corresponding length of time. As indicated by the increased uptake of thymidine and the Feulgen reaction, the hepatic cells, including those in the wall of fatty cysts as well as in the elongated nuclei of the cells in the fibrous stroma strands seen in the cirrhotic livers of the choline-deficient animals, underwent marked proliferative activity. Accordingly, fibroblastic proliferation as well as condensation of stroma would materially account for the fibrous tissue development in nutritional cirrhosis of man and experimental animals, although the present experiments are not entirely conclusive in this respect. M. SILBERBERG

Regeneration in Fatty Livers and Cirrhosis. Autoradiographic Study Using Tritiated Thymidine. R. A., MacDonald, R. Schmid and K. Mallory, *Arch. Path.*, 69: 175, 1960.

Cirrhosis of the liver was produced in male rats of the Sprague-Dawley strain fed a choline-deficient diet. The findings were compared with those seen in control animals fed a complete diet or the deficient diet supplemented subsequently with 2 per cent choline. The observations were extended for periods varying from seven days to fourteen months, and autoradiographic studies of the liver were made with the tritiated thymidine technic. The investigations disclosed that fatty metamorphosis of the liver parenchyma preceded the development of cirrhosis, and that new liver cells formed in association with or as a consequence of the fatty change. Slight fatty metamorphosis was accompanied by limited new formation of liver cords; advanced fatty change was associated with marked liver cell regeneration. M. SILBERBERG

The nutritional versus viral etiologies for hepatic abnormalities noted in the African Negro remains an unresolved problem.

Liver Disease in the South African Bantu. A Review of Liver Biopsies from 262 Bantu Patients. J. Higginson and K. F. Keeley. *Gastroenterology*, 38: 332, 1960.

In a study of 262 Bantu subjects, which included liver biopsy, no definite evidence was found that nutrition was an important cause of liver disease in this African group. The exception to this was two patients with kwashiorkor who had fatty metamorphosis of the liver. In patients with evidence of pellagra and scurvy, the types of liver disease and their frequency were no different than that in other groups. Nine subjects in this series had veno-occlusive disease, presumably due to senecio poisoning. Twelve patients had hepatitis, presumably of viral origin. In eighteen subjects, postnecrotic cirrhosis was found and this was presumed to be due to non-icteric viral hepatitis. Siderotic (pigmentary) cirrhosis was noted in nine subjects. These last two groups constituted the great bulk of patients with clinical evidence of liver disease. The amount of alcohol consumed by the subjects studied could not be stated definitely but was presumed to be moderate.

This study is significant in that it emphasizes the importance of postnecrotic cirrhosis presumably due to a previous episode of viral hepatitis as a causative factor in a large number of patients with cirrhosis in South Africa. The relative frequency in which non-icteric forms of this infection occur is still unknown. The relationship between malnutrition and high iron intake in Bantu subjects to liver disease is perhaps now placed in a minor role. J. B. HAMMOND

Nutritional Siderosis in Ghana. G. M. Edington. *Central African J. Med.*, 5: 186, 1959.

In a series of 728 unselected necropsies, liver siderosis was found in 44 per cent of African males and 34 per cent of females. The condition, attributable to a high dietary intake of iron, has been recorded from many parts of Africa. Histochemical tests indicated that in most cases the patients had a reticuloendothelial iron deposition resulting from nutritional siderosis and that severe reticuloendothelial siderosis could occur without cirrhosis or pancreatic lesions. Nevertheless, cases in which there was a predominantly parenchymatous distribution of iron also occurred, suggesting a different mechanism of iron metabolism with a harmful tissue reaction. Thus, twenty-two patients undergoing postmortem examination showed heavy hepatic iron pigmentation, heavy deposits of iron were also found in the pancreas, while liver biopsy in forty-four patients with diabetes mellitus revealed hemochromatosis in four. R. E. BERNSTEIN

MAGNESIUM METABOLISM

The divalent cation, magnesium, is primarily an intracellular substance sharing some of the properties of calcium and potassium. In plasma, its normal concentration is 1.5 to 2 mEq. per L.; about 98 per cent of the body pool of 28 gm. is intracellular. Hypomagnesemia occurs in alcoholism, cirrhosis of the liver and malabsorption states; hypermagnesemia may be found in renal failure.

Magnesium Metabolism in Rabbits Using Mg^{28} as a Tracer. J. K. Aikawa, E. L. Rhoades, D. R. Harms and J. Z. Reardon. *Am. J. Physiol.*, 197: 99, 1959.

Tracer doses of Mg^{28} were used to study the metabolism of parenterally administered magnesium. Plasma

clearance was rapid, and accumulation of radioactivity in bone started within two hours. Of the tissues studied, skin and muscle showed the lowest concentrations of Mg^{28}. In most tissues the ratio of tissue to serum radioactivity became fairly constant after eighteen hours. The values for the exchangeable body pool of magnesium, calculated from the specific activities of urine specimens obtained between eighteen and twenty-four hours, approximated the carcass content of magnesium. During starvation the renal excretion of endogenous magnesium amounted to 61.7 mEq. per kg. of weight loss. AUTHORS

Magnesium Turnover in the Human Studied with Mg^{28}. L. Silver, J. S. Robertson and L. K. Dahl. *J. Clin. Investigation*, 39: 420, 1960.

Magnesium functions as an activator for many *in vitro* enzyme systems and has been thought to be involved perhaps in such disease states as atherosclerosis, myxedema, hyperthyroidism and acute alcoholism. However, compared to other major ions in the body, magnesium is relatively inert. Studies with Mg^{28} were performed on ten adults. The observations indicate that there are at least three compartments in the body pool of magnesium turning over with half-times of one, three and fourteen to thirty-five hours, respectively. Probably 25 to 50 per cent of the magnesium has a turnover rate of less than 2 per cent per day.

The authors conclude that the gastrointestinal absorption of magnesium ion is very limited. Following intravenous administration the isotope appears in the urine rapidly, but only insignificantly in the stool. Most of the magnesium in the stools is of exogenous origin. The authors caution that the data are based on single experiments in two persons. Nevertheless, the observations are an important contribution to our understanding of this element. S. O. WAIFE

Magnesium deficiency produces certain clinical manifestations resembling hypokalemic alkalosis as well as those of hypocalcemia. In hallucinated or irritable patients with an alcoholic background or severe liver disease, a plasma magnesium determination is warranted.

The Magnesium-Deficiency Tetany Syndrome in Man. B. L. Vallee, W. E. C. Wacker and D. D. Ulmer. *New England J. Med.*, 262: 155, 1960.

Five patients suffering from severe malnutrition caused by either chronic alcoholism or intestinal malabsorption exhibited a tetany as shown by either spontaneous carpopedal spasm or positive Chvostek and Trousseau signs. Three of the patients had generalized convulsions. Athetoid motion of the extremities and hyperactivity to mechanical and auditory stimulation were also noted in some of the patients. All five subjects had low serum magnesium values compared to the normal level of 2.0 ± 0.16 (S.D.) mEq. per L. When the serum level was 0.60 to 1.33 mEq. per L. tetany was maximal. Calcium, sodium and potassium levels of the serum were normal. Prompt and dramatic relief of tetany occurred when the magnesium concentration of the serum was restored to normal by the intramuscular injection of magnesium sulfate.

The magnesium tetany syndrome is indistinguishable from hypocalcemic tetany. To differentiate between the two syndromes it is necessary to measure the serum levels of the two ions. This paper is important since it is the first to establish with any certainty the symptoms of magnesium deficiency in man.

M. W. BATES

PYRIDOXINE IN NUTRITION

The exact requirements of vitamin B_6 have not been determined. Based on experimental deficiency studies in adults using a pyridoxine antagonist it seems likely that 1 to 2 mg. daily of the vitamin should be sufficient. The needs for infants can be supplied by milk which contains 100 μg. per L. The heat-spray evaporation of milk has led to a serious reduction of pyridoxine content of these products in the past.

The Effect of Heat on the Vitamin B_6 of Milk. I. Microbiological Tests. M. E. Gregory. *J. Dairy Res.*, 26: 203, 1959.

Attention was given to the three principal analogues of vitamin B_6 in milk—pyridoxine, pyridoxamine and pyridoxal—and the specificity with which the several organisms used in microbiological analyses measure the different forms of vitamin B_6. Numerous data are reported showing organism growth on the different analogues.

Human milk was found much below cow's milk in vitamin B_6 activity: in terms of μg. per ml., pyridoxine 0.0 and 0.05; pyridoxamine 0.004 and 0.04; pyridoxal 0.004 and 0.45. Pyridoxal accounts for the major portion of the vitamin B_6 activity in raw milk. On heating and storage a portion is converted to pyridoxamine, and a portion is destroyed. FRANK E. RICE

The Effect of Heat on the Vitamin B_6 of Milk. II. A Comparison of Biological and Microbiological Tests of Evaporated Milk. M. K. Davies, M. E. Gregory and K. M. Henry. *J. Dairy Res.*, 26: 215, 1959.

Biological assay methods using rats and chicks showed that the three principal analogues of vitamin B_6—pyridoxine, pyridoxamine and pyridoxal—were equally active. Vitamin B_6 activity by biological methods agrees with microbiological determinations when applied to raw milk. However, with milk processed and stored, the differences were statistically significant, biological methods giving higher results. An "evaporated milk" prepared by the authors from a local milk supply showed a loss of 45 to 70 per cent of vitamin B_6 activity on processing and a further 30 per cent loss on storage. No data are reported on brands of commercial evaporated milk. FRANK E. RICE

The Retention of Vitamin B_6 in Meat During Cooking. C. H. Lushbough, J. M. Weichman and B. S. Schweigert. *J. Nutrition*, 67: 451, 1959.

Meat is a major dietary source of vitamin B_6. Knowledge of the extent to which this vitamin is retained in cooking is important. Vitamin B_6 occurs in several forms (pyridoxine, pyridoxal and pyridoxamine) which have varying biological activities for different experimental animals and organisms. The authors believe that the low vitamin B_6 figures obtained in other laboratories on cooked meats are to be explained by failure to take this into account. In this study, both a microbiological yeast (S. Carlsbergensis) assay and a rat bioassay were employed.

The investigators report a number of tests made to determine the influence of various factors on the vitamin B_6 assay methods. Autoclaving was without significant effect on the vitamin B_6 activity of a pyridoxine-HCl standard solution, but it appeared to destroy about a third of the pyridoxal-HCl solution. The response of the vitamin B_6-depleted rat to equimolar amounts of pyridoxine-HCl, pyridoxal-HCl and pyridoxamine-$(HCl)_2$ was found to be approximately equal. In the microbiological assay, however, the pyridoxal-HCl curve was substantially lower than the others.

On the meat samples, the microbiological assay method yielded results approximately half as large as those determined by rat assay, both in uncooked and cooked meats. But cooking losses percentagewise were about the same by both methods. The retention of vitamin B_6 in cooked meats ranged from 42 to 67 per cent with an average of about 54 per cent.

The drippings from cooked meat carried away relatively small proportions of vitamin B_6, varying from 1 per cent for fresh ham to 13 per cent for leg of lamb.

FRANK E. RICE

Pyridoxine deficiency has been shown to produce convulsions, muscular twitching and irritability with growth failure and anemia in infants and children. In adults, experimental deficiency is associated with mental depression, irritability and somnolence; seborrheic derivatives, glossitis, conjunctivitis and peripheral neuritis have been noted.

Pyridoxine Deficiency and Epilepsy. M. Calvario. *Acta Vitaminol.*, 1: 23, 1958.

The finding of pyridoxine deficiency has been confirmed in about half of the epileptic patients who have been studied. The usefulness of the administration of this vitamin is emphasized, as it might raise the magnesium blood content, promote an increased production of glutamic acid in the cerebral tissue, and bring about a higher concentration of potassium ions in the brain.

AUTHOR

Association of Pyridoxine Deficiency and Convulsions in Alcoholics. A. M. Lerner, L. M. DeCarli and C. S. Davidson. *Proc. Soc. Exper. Biol. & Med.*, 98: 841, 1958.

Pyridoxine deficiency, shown by the excessive urinary excretion of xanthurenic acid after an oral dose of 10 gm. of *dl*-tryptophan, was demonstrated in three of five patients who had convulsions after an acute alcoholic episode and who showed clinical signs of other vitamin deficiencies. Chronic alcoholics who had not had convulsions and those with idiopathic epilepsy gave a normal response to the tryptophan loading test as did the three patients referred to earlier after a single intramuscular injection of 100 mg. of pyridoxine.

G. WALKER

Effect of Pyridoxine in Acute Alcoholic Intoxication. H. Newman and M. E. Smith. *Proc. Soc. Exper. Biol. & Med.*, 100: 258, 1959.

The intravenous injection of 100 mg. pyridoxine had no effect on the clinical condition or the blood alcohol level in dogs given a standard intravenous infusion of 3 gm. of ethanol.

This work was carried out because results obtained in man under less precisely controlled conditions suggested that pyridoxine antagonizes the pharmacological effects of ethanol.

G. WALKER

Effects of Different Vegetable Fats on the Cardiac Damage Caused by Pyridoxine Deficiency. E. Mascitelli-Coriandoli, R. Boldrini and C. Citterio. *Experientia*, 15: 76, 1959.

Cardiac hypertrophy and a reduction in transamination are shown by animals when vitamin B_6 is lacking in the diet. Hypertrophy is attributed to the hypertension caused by pyridoxine deficiency, while the change in transamination is due to the pyridoxine, in the form of pyridoxalphosphate, being the prosthetic group of transaminases. This picture is said to be remarkably similar to one observed in human arteriosclerosis, and because of relationships said to exist between pyridoxine and lipid metabolism the following study was performed.

One hundred and forty-four rats were divided into twelve groups of twelve, each on a diet with no vitamin B_6 to which a vegetable fat had been added. After four weeks on the diet, the hearts were removed and tested for lipid and pyridoxalphosphate contents. The greatest degree of cardiac enlargement was obtained when cocoa butter and coconut oil were used, the least enlargement from soybean and linseed oil. The lower the iodine value of the fat, the greater the increase in cardiac weight. The only exception was cottonseed oil, where the hypertrophy effect was less than with corn oil although the iodine value of the former is lower. In general, these findings apply to the lipid content of the heart tissue.

On the other hand, the pyridoxalphosphate content was highest in the rats administered soybean or wheat germ oil and lowest when olive oil was added. While cardiac hypertrophy and the lipid content of the heart

are related to nonsaturated fatty acid content of the oil, no similar relation exists in respect to the enzyme concentration. According to the authors, linseed oil, which has the highest iodine value of those tested, appears to lower the pyridoxalphosphate contents to a greater extent than does cottonseed, wheat germ or soybean oil, all of which have a higher linoleic acid content although the iodine values are lower. S. O. WAIFE

The Development and Cure of Pyridoxine Deficiency Symptoms in Weanling Mice. L. Mirone and C. D. Jackson. *J. Nutrition*, 67: 167, 1959.

Vitamin B_6 deficiency in animals is exhibited by various symptoms. Among other indications that have been observed are those related to the hematologic picture. In this study, particular attention was given to such changes as hemoglobin, erythrocyte and leukocyte values.

Twenty-one day old mice, male and female, were used. A pyridoxine-deficient diet was employed with and without the addition of deoxypyridoxine, which possesses antipyridoxine activity and thus effects a more rapid and complete pyridoxine depletion.

Pyridoxine-deficient mice could be restored to normal growth and hematologic levels, and cleared of other clinical B_6-deficiency symptoms, by daily injections of 50 μg. of pyridoxine for twenty days, whereas injections of vitamin B_{12}, folic acid or saline, singly or in combination, had no effect.

The authors state that their findings are "in agreement with the many reports that vitamin B_{12} is effective in pernicious anemia only and folic acid in macrocytic anemias other than pernicious anemia."

Deoxypyridoxine in the rations definitely hastened the development of symptoms of vitamin B_6 deficiency. None of the variables investigated was significantly affected by sex. FRANK E. RICE

Hereditary Hypochromic Anemia with Transfusion Hemosiderosis Treated with Pyridoxine. R. C. Bishop and F. H. Bethell. *New England J. Med.*, 261: 486, 1959.

A case study is presented in which a therapeutic response to pyridoxine was found in a man with hereditary hypochromic anemia and transfusion hemosiderosis. When the patient was examined it appeared that the hemoglobin formation and production of red blood cells was abnormally low. The patient was not pyridoxine-deficient since tryptophan metabolism was normal and serum transaminase levels elevated. When pyridoxine (100 mg. daily) was administered a reticulocytosis occurred and the patient was able to maintain his hemoglobin concentration without transfusion. The authors suggest that the large amounts of iron found in the tissues in some way prevented the functioning of pyridoxine as a coenzyme in heme synthesis. After the administration of pyridoxine this inhibition of heme synthesis was overcome and the patient was able to produce red blood cells. These cells, however, were hypochromic and it was thought that this anemia was due to a genetic defect. M. W. BATES

Vitamin B_6 Deficiency in Blood Diseases. G. Gehrmann. *German M. Month*, 4: 336, 1959.

Deficiency of vitamin B_6 in animals and human infants has been shown to produce a hypochromic anemia. The clinical significance has only recently been recognized, with the observation of cases of spontaneous pyridoxine deficiency anemia in man. This condition is proved by a tryptophan tolerance test and pyridoxine is the specific cure. The author has used the xanthurenic acid excretion following an oral test dose of tryptophan in his studies. Ten grams of DL-tryptophan is given orally. Fifty milligrams of xanthurenic acid excretion is taken as the upper limit of normal per twenty-four hours.

The test was carried out in 159 patients, and a vitamin B_6 deficiency was demonstrated in twenty-three. There were no characteristics in the blood or in the skin by which a clinical diagnosis could have been made. The interesting feature of his results was that in most of the anemic patients the vitamin B_6 deficiency state was found to be due to malignant disease. Thus of twelve patients with malignancy and anemia, nine showed an abnormal reaction to the tryptophan test. These patients were then treated with pyridoxine. In only two was the hemoglobin level rapidly restored to normal. In only one of the other patients with malignancy, but without anemia, was such a deficiency detected.

The anemia of pyridoxine deficiency has certain features in common with pernicious anemia, namely, a high serum iron, hemosiderosis, hyperplastic marrow and neurologic complications. However, none of four patients with pernicious anemia showed vitamin B_6 deficiency by the tryptophan test.

No relation could be found between this deficiency and the type, duration or rate of growth of the malignant tumor or with the distribution and extent of the metastases. The author believes it is probable that metabolic disturbances caused by the tumor growth are responsible for the anemia and for the concomitant pyridoxine deficiency.

Vitamin B_6 deficiency as determined by the tryptophan test, was noted in two of four patients with acute leukemia, two of six with chronic myeloid leukemia, two of three with lymphosarcoma, and two of three with multiple myelomas. However, in a wide variety of diseases associated with blood dyscrasias, results of the test were normal. S. O. WAIFE

VITAMIN B_{12} AND INTRINSIC FACTOR

The absorption of vitamin B_{12} is markedly reduced or absent in the absence of a heat-labile protein enzyme known as intrinsic factor produced by the gastric mucosa. This factor exhibits a high degree of species specificity; hog and human instrinsic factors have similar activity in man while they decrease vitamin B_{12} absorption in rats.

The principal portion of the intestine for vitamin B_{12}-intrinsic factor complex absorption is the ileum.

Factors Controlling B_{12} Uptake by Intestinal Sacs In Vitro. E. W. Strauss and T. H. Wilson. *Am. J. Physiol.*, 198: 103, 1960.

Sacs of everted small intestine were incubated in bicarbonate-saline containing radioactive vitamin B_{12} with or without a source of gastric intrinsic factor (IF). In both the hamster and guinea pig the lowest ileum was most active in vitamin B_{12} uptake in the presence of intrinsic factor, the upper jejunum showing little or no uptake. Low temperature and anaerobic conditions completely abolished the stimulatory effect of IF on vitamin B_{12} uptake. Intrinsic factor did not bind to the intestinal wall in the absence of vitamin B_{12} (even in the presence of calcium ion) as the IF activity could be completely removed by gentle washing of the tissue. The vitamin and intrinsic factor must be present together to cause intestinal uptake of vitamin B_{12}.

AUTHORS

Some Species Differences in the Intrinsic Factor Stimulation of B_{12} Uptake by Small Intestine In Vitro. T. H. Wilson and E. W. Strauss. *Am. J. Physiol.*, 197: 926, 1959.

Sacs of everted small intestine from a variety of animals were incubated in bicarbonate-saline containing vitamin B_{12} with and without intrinsic factor (IF). Vitamin B_{12} uptake by rat intestine was stimulated only by its own intrinsic factor. Guinea pig ileum responded to all intrinsic factors tested (guinea pig, rat, hog, hamster, human being and rabbit). The intestines of hamster and rabbit were intermediate in specificity, responding to some, but not all, of the IF preparations. Species differences occur in both the intestine and intrinsic factor preparations. The guinea pig ileum was suggested as a possible assay for both hog and human IF.

AUTHORS

Absorption, Excretion and Tissue Distribution of Oral Cyanocobalamin in Rabbits. H. L. Rosenthal. *Am. J. Physiol.*, 197: 1048, 1959.

The appearance of radioactivity in plasma, following an oral dose of Co^{60} cyanocobalamin, reached maximum values after seven hours. The plasma activity during the succeeding twenty-six day experimental period decreased in a logarithmic fashion which was resolved into three components with biological half-lives of twenty-nine hours, 7.1 days and 15.8 days. The 7.1 day half-life component is significantly longer than the four day half-life found in rabbits following intramuscular or intravenous administration of Co^{60} cyanocobalamin. This indicates a different, more stable, protein-cyanocobalamin complex formed during absorption. The excretion of radioactivity in urine and feces following oral dosage of Co^{60} cyanocobalamin demonstrates the limited absorption of cyanocobalamin from the gut of rabbits. The urinary excretion of the radioactive dose increased from 2.2 to 20.7 per cent in animals previously given an injection of nonradioactive cyanocobalamin, and liver tissue retained more radioactivity following supplementation with nonradioactive cyanocobalamin. It is concluded that the cyanocobalamin absorption mechanism of rabbits is similar to that of human subjects and other laboratory animals.

AUTHORS

The existence of autoantibodies specific for certain tissues has been demonstrated in a variety of diseases such as thyroiditis and hepatitis. A causal relationship has been proposed in which the antibody is regarded as a damaging agent for the tissue involved. Is it possible that such autoantibodies exist in patients with pernicious anemia to inhibit the action of intrinsic factor and to damage the cells responsible for its formation?

Inhibition of Intrinsic Factor by Pernicious Anaemia Sera. K. B. Taylor. *Lancet*, 2: 106, 1959.

The serums of nineteen patients with pernicious anemia were tested. All subjects had been selected on the basis of absorption tests of vitamin B_{12} labeled with radioactive cobalt (Co^{60}) using the fecal excretion method. A standard test dose of 0.5 mg. of Co^{60} vitamin B_{12} was injected alone or with 50 mg. of hog intrinsic factor (IF). The IF had already been mixed with 45 to 55 ml. of human serum to be tested. In nine of the nineteen serums tested, the percentage absorption of vitamin B_{12}, when given with the serum-IF mixture, was lower than the mean for normal control serums for the range imposed by twice the standard deviation (16 per cent). In three such patients, the oral administration of hydrocortisone reduced the inhibitory effect, but did not increase the capacity to absorb vitamin B_{12}. Steroid administration has been well known to reduce antibody activity. The authors excluded inhibition due to blood group isoagglutinins, since although the hog IF contained A substance, inhibitory serums were found in patients with blood groups O, A or B. Inhibition of IF was produced by serums of treated and untreated patients. The data suggest, but do not establish, that the activity of hog IF was inhibited by an antibody in the serums of patients with pernicious anemia, but not in the serums of control subjects.

E. COHEN

Elevated vitamin B_{12} levels in serum of patients with liver disease has been attributed to the release of the vitamin from damaged hepatic cells. In leukemia, the elevation may be due to the increased vitamin B_{12}-binding capacity of serum.

The Plasma Vitamin B_{12} Binding Substance. I. Its Detection in the Seromucoid Fraction of Plasma from Normal Subjects and Patients with Chronic Myelocytic Leukemia. I. B. Weinstein, S. M. Weissman and D. M. Watkin. *J. Clin. Invest.*, 38: 1904, 1959.

The vitamin B_{12} content of the seromucoid fraction of plasma from normal subjects and patients with chronic

myelocytic leukemia was studied. The binding substance appears to be present in this fraction suggesting that it is a glycoprotein. The data also implied that the elevated serum vitamin B_{12} levels seen in chronic myelogenous leukemia are associated with an increase of specific vitamin B_{12}-binding glycoprotein.

S. O. WAIFE

Vitamin B_{12} Stores in the Human Body. II. Vitamin B_{12} Content of Tissues in Health and Diseases. C. I. Jhala and R. K. Gadgil. *Ann. Biochem. & Exper. Med.*, 19: 293, 1959.

The vitamin B_{12} content of normal and diseased tissues was determined at autopsy. Raised serum levels have been observed in cases of hepatic damage and are associated with lowering of the vitamin B_{12} content of the liver. There was a decrease in the content in different areas of the diseased liver in proportion to the hepatic damage. This suggests possible release of the vitamin from the liver. The vitamin content of the kidney and spleen remain normal in hepatic damage.

S. O. WAIFE

The treatment of pernicious anemia with parenterally administered vitamin B_{12} at weekly, then monthly, intervals is entirely satisfactory. Nevertheless other therapeutic programs are constantly advocated.

Maintenance Treatment of Pernicious Anaemia by Massive Parenteral Doses of Vitamin B_{12} at Intervals of Twelve Weeks. J. D. Kinloch. *Brit. M. J.*, 1: 99, 1960.

The usually recommended treatment for pernicious anemia is parenterally administered vitamin B_{12} given every two to four weeks. This is tedious for the patient. This paper describes an attempt to lengthen this period to three months by giving doses of about 1 mg. (1,000 μg.).

One hundred patients were included in the trial. Of these eighty-seven were well maintained over a period of two years. The remaining thirteen showed unsatisfactory red cell levels after nine to sixteen months; eleven of these showed improvement when the dosage of vitamin B_{12} was increased to 1 mg. monthly.

It is concluded that while treatment at three-month intervals is suitable for most patients it cannot be recommended as a routine measure. This seems an unduly pessimistic conclusion; since progress is easily judged, it would be well worth giving all patients an opportunity, in the first instance, of having the less frequent treatment. F. E. HYTTEN

Oral Treatment of Pernicious Anaemia with Vitamin B_{12} and Desiccated Hog Duodenal Extract. E. K. Blackburn, G. H. Spray, H. T. Swan, G. R. Tudhope and G. M. Wilson, *Brit. M. J.*, 2: 535, 1959.

Although oral administration of a substance with intrinsic factor activity is the rational approach to the treatment of pernicious anemia it has not yet been proved as consistently effective as parenterally administered vitamin B_{12}.

Thirteen patients with Addisonian anemia who had had normal blood pictures for a year with parenterally administered vitamin B_{12} were transferred to treatment with an oral preparation, Biopar,® containing 6 μg. of vitamin B_{12} and 30 mg. of an intrinsic factor preparation. Nine additional patients with previously untreated pernicious anemia, who showed a good initial response to Biopar, were included in the study.

All patients were studied in detail for up to five years. The blood level of vitamin B_{12} remained low with the oral treatment and two patients had a relapse. Skin tests for sensitivity to hog duodenal mucosa showed no relationship to the inability to respond to Biopar. It is concluded that oral treatment of pernicious anemia is unreliable. F. E. HYTTEN

Intestinal Absorption of Liver-Bound Radiovitamin B_{12} in Patients with Pernicious Anaemia and in Controls. P. G. Reizenstein and W. Nyberg. *Lancet*, 2: 248, 1959.

Two groups of subjects, one of nine healthy volunteers and two hospital patients and the other of ten patients with pernicious anemia, were given raw ground hog or calf liver containing radioactive vitamin B_{12} on one occasion and a drink containing radioactive crystalline vitamin B_{12} on another occasion at least eight days apart.

Both groups absorbed more of the liver-vitamin B_{12} complex, and the hematologic response was greatest when most radioactivity was absorbed. Some 10 per cent of the radioactivity in the liver was absorbed.

It is suggested that the nondialysable complexes of vitamin B_{12} occurring in liver may be more readily absorbed, perhaps without the need for intrinsic factor, than crystalline cyanocobalamin. (This is an important paper in the field.) F. E. HYTTEN

Vitamin B_{12} Absorption During Pregnancy. A Controlled Study. R. P. Gotchel and B. F. Lovett. *Am. J. Obstet. & Gynec.*, 79: 113, 1960.

Forty-six women received oral vitamin B_{12} or a placebo from the first or early part of the second trimester of pregnancy until the third day postpartum. Of these, twenty-two received 25 μg. of vitamin B_{12} and 11 gm. of D-sorbitol per day. Plasma vitamin levels were determined by microbiologic assay. As expected, the plasma vitamin B_{12} levels were higher in the group receiving the vitamin supplement than in the control, or placebo, group.

Since dissimilar subjects were used and patients did not serve as their own controls, one can hardly be impressed with these results. Obviously, nothing can be said about the possible effectiveness of D-sorbitol in altering vitamin B_{12} levels. No significant change in the blood picture was reported. The article also does not give the standard deviation or standard error of the blood levels, merely average responses. S. O. WAIFE

Effects on the Vitamin B_{12} Content of the Blood of Doses of Cyanocobalamine Given Orally, Rectally and Parenterally. H. Gounelle and J. Richard. *Le Sang*, 29:252, 1958.

Intramuscular administration assures a considerable and very rapid effect on the serum vitamin content, unlike administration through the digestive tract, either oral or rectal, which entails massive waste. The effect of rectal administration of vitamin B_{12}, with or without penicillin, is of very limited value. H. GOUNELLE

A Comparison of Maternal and Foetal Folic Acid and Vitamin B_{12} at Parturition. H. Baker, H. Ziffer, I. Pasher and H. Sobotka. *Brit. M. J.*, 1:928, 1958.

Folic acid and vitamin B_{12} levels were estimated in the cord blood of 113 infants at delivery, and in venous blood from their mothers.

The median values for serum vitamin B_{12} were 190 $\mu\mu$g. per ml. in the mother and 390 $\mu\mu$g. per ml. in the infant; for folic acid the respective levels were 7.5 mμg. per ml. and 40 mμg. per ml. The individual variation was, however, very large and there was considerable overlap; in some cases the levels in maternal blood were higher than those in infant blood.

This evidence is interpreted as indicating a high fetal avidity for vitamin B_{12} and folic acid, depleting the maternal stores, but considerably more information will be needed about maternal and fetal requirements and metabolism of these substances before this simplified explanation can be accepted. F. E. HYTTEN

The effect of iron and ascorbic acid upon the production of intrinsic factor and the reduction of vitamin B_{12} absorption may be a fruitful area of investigation.

Interrelation of Vitamin B_{12} and Iron. E. V. Cox, M. J. Meynell, R. Gaddie and W. T. Cooke. *Lancet*, 2:998, 1959.

This is a study of thirteen subjects in whom an iron deficiency normoblastic anemia was associated with a serum vitamin B_{12} level of 100 $\mu\mu$g. per ml. or less. In all but one patient the vitamin B_{12} level rose to normal as the anemia was corrected with iron therapy. The return was slow, with low levels persisting after at least ten weeks of treatment, except when the anemia was associated with chronic alimentary bleeding. In such patients there was a quick return, within two or three weeks, to normal blood levels of vitamin B_{12}. It is suggested that the phenomenon is due to poor vitamin B_{12} absorption which is corrected by iron treatment The generally accepted relationship between low levels of vitamin B_{12} in the blood and bone marrow changes is not universal. F. E. HYTTEN

An Inter-relationship between Ascorbic Acid and Cyancobalamin. E. V. Cox, R. Gaddie, D. Matthews, W. T. Cooke and M. J. Meynell. *Clin. Sc.*, 17:681, 1958.

A relationship between ascorbic acid and vitamin B_{12} has been noted on a number of occasions; for example, plasma vitamin C levels are reduced in pernicious anemia and vitamin C may correct megaloblastic erythropoiesis in scurvy.

The subjects of the present investigation were thirty-two normal persons, twelve with pernicious anemia in relapse, seven with adult celiac disease and fifteen with regional ileitis, seven of whom were vitamin B_{12} deficient. The effect of vitamin B_{12} administration on plasma vitamin C levels before and after the intravenous injection of 250 mg. of sodium ascorbate was studied. Subjects with low serum vitamin B_{12} levels had a generally lower plasma vitamin C level than normal control subjects and injected vitamin C disappeared more quickly. After vitamin B_{12} therapy this abnormal vitamin C metabolism was corrected in the patients with pernicious anemia and improved in those with celiac disease, but there was no change in those with regional ileitis. In regional ileitis the lack of vitamin B_{12} was attributed to the activity of the underlying disease; there was a marked correlation between the levels of seromucoid and resting plasma ascorbic acid.

F. E. HYTTEN

The absorption, utilization, turnover and destruction of vitamin B_{12} are affected by many factors some of which are known, such as the binding by intrinsic factor, serum protein and tissue cells. However, there are many gaps in our knowledge of the metabolism of this vitamin apart from its physiologic effects.

Calculations on Vitamin B_{12} Turnover in Man. With a Note on the Maintenance Treatment in Pernicious Anemia and the Radiation Dose Received by Patients Ingesting Radioactive Vitamin B_{12}. R. Gräsbeck. *Scandinav. J. Clin. & Lab. Invest.*, 11:250, 1959.

Based on certain assumptions and mathematical calculations, the author concludes that the percentage of the vitamin B_{12} pool excreted per day ranged from 0.15 to 0.3 per cent and the biologic half-life from 230 to 460 days. On the basis of these calculations, a suitable daily maintenance dose would be one which causes the retention of 10 μg. of vitamin B_{12} per day, but it is not known whether such treatment has advantages over the conventional one, because normal subjects might absorb more than they actually need. Although the minimum effective maintenance dose is of the order of 0.5 to 1 μg. a day, this has led to the conclusion that the minimum combined utilization, destruction and loss of absorbed vitamin B_{12} is of the same order of magnitude. However, the author believes that this dose cannot be considered identical with the turnover under normal conditions because his calculations indicate that the "requirement" of vitamin B_{12} is of the order of 0.4 to 0.5 μg. per day. This paper cannot easily be abstracted; it should be seen in the original by those interested. S. O. WAIFE

Persistence of the Antipernicious Anemia Action of Vitamin B_{12}. E. Aron and Y. Cargnelutti. *Presse méd.* 67: 1345, 1959.

Two patients with pernicious anemia were successfully treated with vitamin B_{12}, after which they received no further treatment. Both returned to the hospital in a state of serious relapse, one after five years and the other after four years. These observations are evidence of the great duration of vitamin B_{12} storage in the liver after a course of treatment, and explain why pernicious anemia in relapse is now such a rare disease.

H. GOUNELLE

Instability of Radioactive Vitamin B_{12}. E. L. Smith. *Lancet*, 1: 387, 1959.

Radioactive vitamin B_{12} is now widely used both for diagnosis in anemia and for more fundamental studies, but since its use assumes that the radioactivity measures the vitamin it is important that no other radioactive material is present.

In these careful experiments it is shown that labeled vitamin B_{12} is liable to decompose on storage; the rate of decomposition is unpredictable and shows no evident correlation with specific activity, nature of the cobalt isotope or the concentration. When decomposition has occurred measurements of radioactivity are no longer a reliable indication of vitamin B_{12} content and stocks should be tested at intervals of not more than a month.

F. E. HYTTEN

Vitamin B_{12} Deficiency and Amino-Aciduria. S. K. Gokhale and B. D. Punekar. *Ann. Biochem. & Exper. Med.*, 19: 159, 1959.

Better growth responses were observed in rats receiving vitamin B_{12}, confirming the previous findings that vitamin B_{12} promotes anabolic processes.

Vitamin B_{12} deficiency in rats has been found to cause aminoaciduria, characterized by increased excretion of free as well as combined *a*-amino-nitrogen in the urine.

Microbiologic estimations have shown that the amino acids excreted in larger amounts in vitamin B_{12} deficiency are glutamic acid, lysine, glycine, phenylalanine, tryptophan and methionine.

It is suggested that the aminoaciduria in vitamin B_{12} deficiency is a result of the increased breakdown of tissue proteins and that vitamin B_{12} enhances protein biosynthesis by improving the utilization of circulating blood amino acids.

AUTHORS

NOTES ON NIACIN

When administered in large doses, nicotinic acid has been demonstrated to exert a hypocholesterolemic effect in man and in experimental animals. In the latter studies a reduction in the degree of atherosclerosis induced by cholesterol feeding has been observed following the administration of niacin. These effects cannot be produced with niacinamide. While it has been suggested that this action may be the result of anorexia produced by the large dose of niacin, the e is evidence that hepatic cholesterogenesis is decreased by its administration.

Effect of Nicotinic Acid on Experimental Atheromatosis of Rabbits. E. E. Cava, R. W. P. Achor, K. G. Berge, K. G. Wakim, J. E. Edwards, B. F. McKenzie and N. W. Barker. *Proc. Staff Meet. Mayo Clin.*, 34: 502, 1959.

Albino rats were given a high cholesterol diet with and without nicotinic acid. Hypercholesteremia was present in both cholesterol-fed groups but was less marked in the group that also received large doses of nicotinic acid. Furthermore, in this latter group the degree of aromatic atheromatosis was less than in the group receiving cholesterol without nicotinic acid. Administration of large doses of nicotinic acid orally to animals fed the control diet produced no detectable toxic effects upon the hepatic or renal parenchyma, nor did it cause any significant change in the concentration of serum cholesterol. Large doses of nicotinic acid did not prevent the accumulation of lipids in the parenchyma of the liver.

S. O. WAIFE

Influence of Nicotinic Acid on Hepatic Cholesterol Synthesis in Rabbits. H. Schade and P. Saltman. *Proc. Soc. Exper. Biol. & Med.*, 102: 265, 1959.

The addition of 0.5 per cent nicotinic acid to cholesterol-low and cholesterol-supplemented diets fed to rabbits reduced the *in vitro* incorporation of C^{14}-acetate into digitonin-precipitable sterol in liver tissue obtained from the animals. It is suggested that large amounts of nicotinic acid interfere with cholesterol synthesis in the liver by competing for coenzyme A which is necessary for the detoxification of nicotinic acid to nicotinuric acid as well as for the synthesis of cholesterol.

G. WALKER

Occurrence of Fibrinolytic Activity Following Administration of Nicotinic Acid. M. Weiner, W. Redisch and J. M. Steele. *Proc. Soc. Exper. Biol. & Med.*, 98: 755, 1958.

Blood drawn from patients who had received an intravenous injection of 1 to 3 mg. per kg. of nicotinic acid showed increased fibrinolytic activity. The effect was first seen in specimens taken after four minutes and was maximal ten to fifteen minutes after the injection. It was not found when nicotinic acid in concentrations up to 500 mg. per L. was added *in vitro*.

Large doses (of the order of 6 gm. daily) of nicotinic acid have been recommended in the treatment of atherosclerosis because of the hypocholesterolemic effect of the drug. Although the effect of oral administration was not studied, the results of this paper suggest that activation of a fibrinolytic system might be a further justification for nicotinic acid therapy in patients with atherosclerosis.

G. WALKER

As in the case of other vitamins of the B complex, an interrelationship between niacin and the adrenal cortex

can be postulated on the basis of the interesting case subsequently described.

Addisonian Crisis Due to Pellagra. F. D. Rosenthal and F. Lees. *Lancet*, 1: 665, 1957.

Collapse and sudden death have long been known to be concomitants of pellagra. A case is presented here of a sixty year old widow who had subsisted on a poor diet and was admitted to the hospital suffering from pellagra. Her serum sodium was 137, potassium 4.6, chloride 97 and alkali-reserve 26 mEq. per L.

After fasting overnight for a barium meal she suffered shock with a blood pressure of 60/0 mm. Hg. The serum sodium had fallen to 130 and the potassium had risen to 7.4 mEq. per L. Serum chloride was 92 mEq. but the alkali-reserve had fallen to only 10 mEq. per L.

The patient's clinical and biochemical recovery is described after treatment with nicotinamide, vitamin B complex, sodium lactate and cortisone. She has since recovered completely. The biochemical features suggest an acute failure of mineralocorticoid metabolism. Reports of selective damage of the zona glomerulosa of the adrenal cortex in pellagra provide a possible basis for this failure. F. E. HYTTEN

The conditioned deficiencies of certain amino acids provoked by an imbalance in dietary intake of these factors may serve as a useful concept in interpreting the amino acid-vitamin relationship discovered in the following study.

Leucine and Pellagra. C. Gopalan and S. G. Srikantia. *Lancet*, 1: 954, 1960.

Pellagra is commonly associated with maize-eaters, and this has been attributed to the low tryptophan content of maize and to the poor availability of nicotinic acid.

Pellagra is uncommon in Coonoor where rice is the staple, and common in Hyderabad where the diets of the poor contain large amounts of millet. Millet has as much tryptophan as rice and more nicotinic acid than either rice or maize, but maize and millet both have a very high leucine content compared to rice.

In both normal persons and those with pellagra, oral administration of 5 gm. L-leucine resulted in an increased urinary excretion of N′-methyl nicotinamide. The same effect was produced by the isocaloric substitution of rice by millet. The oral administration of 20 to 30 gm. L-leucine daily to two subjects with pellagra resulted in a deterioration of their condition which was reversed by nicotinic acid when the administration of leucine was discontinued.

The exact role of leucine in nicotinic acid metabolism is not clear but it is suggested that any excess of leucine in a diet which is marginal in respect of protein may result in depletion of nicotinic acid from the tissues. F. E. HYTTEN

The addition of coffee to the diet of those with pellagra may be a pleasant method of combatting their disease.

Human Studies of Biologic Availability of Niacin in Coffee. G. A. Goldsmith, O. N. Miller, W. G. Unglaub and K. Kercheval. *Proc. Soc. Exper. Biol. & Med.*, 102: 579, 1959.

Coffee when darkly roasted contains appreciable amounts of niacin. The demonstration of increased amounts of N′-methylnicotinamide and its pyridone in the urine of five normal subjects when coffee was added to a diet of known and constant niacin and tryptophane content, shows that it is biologically available to man. The infusion used contained about 3 mg. niacin per 100 ml. That coffee is such an important source of niacin has hitherto been overlooked. G. WALKER

ITEMS OF GENERAL INTEREST

Insulin-Like Activity of Normal and Diabetic Human Serum. P. M. Beigelman. *Diabetes*, 8: 29, 1959.

Using the glucose uptake by epididymal adipose tissue from Wistar rats as a method of insulin assay, the author studied the insulin-like activity in serum from normal young adults, diabetic patients in coma and during treatment. The method is reportedly sensitive to 10 microunits of insulin per ml. Postprandial serum from normal subjects showed insulin-like activity by this method. Serum from seven of eight untreated patients in diabetic coma did not show insulin-like activity while all showed activity after therapy. The author emphasizes that this bioassay measures a total biologic effect, i.e., glucose uptake, and is not specific for insulin. G. HOLLIFIELD

Blood Lipids, Clotting and Coronary Atherosclerosis in Rats Exposed to a Particular Form of Stress. H. N. Uhley and M. Friedman. *Am. J. Physiol.*, 197: 396, 1959.

A group of rats fed a diet high in fat and cholesterol were exposed to a specially designed cage inducing a particular form of stress. At the end of ten months these rats exhibited a greater degree of hypercholesteremia and hyperlipemia than the control animals. In addition, considerably more coronary atherosclerosis was observed in the experimental group than in the control group. AUTHORS

Dietary Treatment of an Infant with Phenylketonuria. F. S. W. Brimblecombe, M. E. R. Stoneman and R. Maliphant. *Lancet*, 1: 609, 1959.

It has been thought that if the blood phenylalanine level could be maintained at normal levels in patients with phenylketonuria, the usual rapid mental deterioration could be arrested. In the case described, a male infant, in whom this condition was first diagnosed at about ten weeks of age while in the hospital with gastroenteritis, was given a special diet from the age of seventeen weeks. The diet contained 75 to 100 calories per kg. daily and 25 mg. per kg. of both phenylalanine and tryptophan. A good deal of variation of diet was needed as the child grew but no details are given; the mother has been responsible for the diet since the child

was twenty-two weeks old. At two and a half years of age the child is apparently normal both intellectually and physically; this lends weight to the suggestion that dietary treatment—begun early enough—would successfully counter this metabolic error.

F. E. HYTTEN

Exudative Enteropathy. Abnormal Permeability of the Gastrointestinal Tract Demonstrable with Labelled Polyvinylpyrrolidone. R. S. Gordon. *Lancet*, 1: 325, 1959.

A condition of "hypercatobolic hypoproteinemia" has been described, characterized by an alteration in plasma-protein metabolism and a severe hypoproteinemia. Nine subjects with this condition were given an injection of I^{131}-labeled polyvinylpyrrolidone. The loss of the I^{131} in the stool was measured in the succeeding ninety-six hours. There was an increasing excretion of these large molecules in rough relationship to decreasing plasma albumin concentration; it is suggested that in this condition protein is lost into the gut. The term "exudative enteropathy" is suggested for the disorder which is a manifestation of various gastrointestinal conditions rather than a disease in its own right.

F. E. HYTTEN

Response of the Blood Glucose to Glucocorticoids in Man. K. M. West. *Diabetes*, 8: 22, 1959.

The hyperglycemic effects of orally administered prednisolone and hydrocortisone were studied in healthy adults in an effort to establish a method for estimating the potencies of glucocorticoids. The magnitude of the hyperglycemic response to a given dose of a glucocorticoid was found to be fairly consistent in the same subject from day to day, despite the large variation in response from subject to subject. Single doses of the drugs studied produced small but consistent elevations of fasting blood glucose levels, but the effects on blood glucose were more pronounced when intravenous glucose tolerance tests were performed. The author believes that this is a simple method for comparing the hyperglycemic effectiveness of glucocorticoids.

G. HOLLIFIELD

Patterns of Fat Excretion in Feces of Premature Infants Fed Various Preparations of Milk. M. Davidson, M.D. and C. H. Bauer, M.D. *Pediatrics*, 25: 375, 1960.

The purpose of the current study was to discern whether the steatorrhea of the prematurely born infant is also accompanied by the excretion of large amounts of saturated fatty acids. Groups of infants were fed relatively isocaloric feedings containing varying amounts of both saturated and unsaturated fatty acid in the fat. The per cent absorption was measured in each case.

The per cent of fat absorbed was not significantly different, irrespective of amount of fat or protein in the diet in three groups in which the source was animal fat. In a fourth group in which corn oil was substituted for animal fat, there was a significantly greater degree of fat absorption. There was a relatively constant output of saturated fatty acid with each of the diets—the per cent of the stool being made up of these acids varying more with the type of diet than with the per cent of fat absorbed. This suggests that the source of fat in the stool of the premature infant is virtually all exogenous in origin.

J. L. DENNIS

Body Composition and In Vitro Synthesis of Lipids by Adipose Tissue of 11-Dehydrocorticosterone-Treated Mice. G. Hollifield and W. Parson. *Am. J. Physiol.*, 197: 105, 1959.

Compound A (11-dehydrocorticosterone) pellets implanted subcutaneously produced increased carcass fat and decreased protein in older mice but not in young mice of the DBA strain. The increased carcass fat is not the result of increased food intake or weight gain since it occurs in the face of a decreased food intake and weight loss and is associated with a decrease in spontaneous running activity. Adipose tissue of mice treated with compound A pellet implants has a greater lipid synthesis from C^{14}-labeled acetate than that from cholesterol-treated control animals. These findings lend support to the idea that compound A causes increased gluconeogenesis and increased insulin production with a resultant stimulation of fat synthesis by adipose tissue.

AUTHORS

The Nature of the Ground Substance Changes in Experimental Lathyrism and Their Effect on Atherogenesis in Cholesterol Fed Rabbits. C. J. Schwartz. *Brit. J. Exper. Path.*, 40: 44, 1959.

Male chinchilla rabbits about six months of age and weighing between 1.2 and 1.8 kg. were fed a diet containing 75 gm. mash enriched with 6 ml. peanut oil and 1 gm. cholesterol per kg. One group of animals received daily subcutaneous injections of 50 mg. beta-aminopropionitrile fumarate until the characteristic skeletal and aortic lesions of lathyrism had developed. The aortic changes, as studied by histochemical methods, consisted of separation of elastic fibers, elastolysis and deposition of increased amounts of metachromatic material in the ground substance. This material was partly PAS-positive. The visceral lesions of lathyrism were accompanied by markedly increased levels of serum hexosamine and mucoprotein indicating possibly increased destruction or depolymerization of the aortic ground substance. Following consumption of the atherogenic diet, lipoidosis and calcification were localized about the lathyrus lesions. Moreover, the severity of the atherosclerotic lesions was markedly intensified by the lathyrism. It is believed that metachromatic mucopolysaccharides give rise to the early tissue changes which subsequently cause the lipoid deposition, fibrogenesis and calcification of the atherosclerotic process.

M. SILBERBERG